ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

MICROBIAL LOAD OF GOAT CARCASSES IN SELECTED EXPORT ABATTOIRS LOCATED AT DEBRE ZEIT AND MODJO TOWNS, EAST SHOA ZONE OF OROMIA REGIONAL STATE

BY

TESFAYE MENGISTU TEKA

JUNE 2007

DEBRE ZEIT, ETHIOPIA

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Tropical Veterinary Public Health.

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ABBREVIATIONS

AMBC	Aerobic Mesophilic Bacteria Count
APC	Aerobic Plate Count
APHA	American Public Health Association
BAM	Bacteriological Analytical Manual
CCPs	Critical Control Points
Ec	*Escherichia coli*
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GHP	Good Hygienic Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Point
ICMSF	International Commission on Microbiological Specification for foods
IMS	Immunomagnetic Separation
IMViC	Indole, Methyl Red, Voges Proskauer, Reaction and Citrate Utilization
ISO	International Organization for Standardization
MPN	Most Probable Number
MRD	Maximum Recovery Diluent
NACMCF	National Advisory Committee on Microbiological Criteria for Foods
NRC	National Research Center
SPSA	Sanitary And Phyto-Sanitory Agreement
TEC	Total Enterobacteriaceae Counts
TVC	Total Viable Counts
WTO	World Trade Organizations

ABSTRACT

A total of 432 carcass and knives swabs and 64 water samples were collected from Selected export abattoirs located at Debre Zeit and Modjo towns of East Shoa Zone of Oromia Regional State to assess the microbial load of goat carcasses, knives and water. The study was conducted from October 2006 to April 2007.Swabs were taken from brisket and peri- anal area, as well as from knives used for ripping, and eviscerations before and after washing. Aerobic mesophlic bacteria count was performed using standard plate count agar at 30^0C and incubated for 72 hours and coliform count was assessed using MacConkey agar No 3 and by incubating samples at 35^0C for 18 hours. The study indicated that dipping knives in knife sterilizer at 82^0C and above reduced the Aerobic mesophilic bacterial load by 99.9% and 55.33-68.41 % for coliforms. Mean aerobic mesophilic bacteria count at brisket and peri-anal area of carcasses decreased after washing in all the abattoirs, except in abattoir "W" where an increased aerobic mesophilic count was detected in the brisket area. The mean coliform count in brisket area of the carcasses increased after washing while it decreased in peri-anal area. These results showed increment of mean coliform count after washing in brisket area and decrement at perianal area. This indicates the dissemination of coliforms from highly contaminated peri anal area to the less contaminated brisket area during the washing of the carcasses in conventional carcass dressing system. Comparison between abattoirs revealed that abattoirs "Y" and "Z" have lower mean bacteria load than abattoirs "W" and "X". Out of the 64 water samples tested 39.06% and 18.75% were aerobic mesophilic and coliform bacteria positive, respectively. Antimicrobial susceptibility test conducted on 20 isolates of *E.coli* strain did not show any resistant to the most commonly used antibiotics.

Keywords: - Aerobic mesophilic bacteria, coliform.

1. INTRODUCTION

Ethiopia earns annually on average about 3 596 398.00 USD through export of fresh meat (FAO, 2005). This increases country's foreign exchange, enables to keep favorable balance of trade and contributes to the improvement of the living standard of the pastoralists and farmers. The pastoralists lose their livestock due to drought almost every two years. The livestock lose is maximized due to lack of market which compel the pastoralists to hold more livestock for a long period of time. Despite the changing trend of pastoralists to hold more camel and goats than cattle and sheep, while the formers can withstand drought better, they still keep these for a long period of time

Meat export abattoirs will be able to curtail the market problem of the pastoralists, if they are given proper technical support through research and development strategy. This strategy enables the meat export abattoirs to meet the international standards along with import requirements of importing countries and follow strictly the microbiological guidelines in order to provide safe, sound and wholesome meat to the consumer of importing countries.

Trade in meat is a risky venture while meat deteriorates when contaminated with microorganisms. Such contaminations result in meat spoilage, infections and intoxications in humans. The former results in severe economic losses and the latter poses hazards to the public health and impedes meat export. International organizations and importing countries have set microbiological limits for meat trade. Failure to comply with the import requirement results in loss of market. Ethiopia as a meat exporting country should strive to produce microbiologically safe meat to secure its place in the international market. Hence there is a need to monitor the microbiological status of export meat in Ethiopia to ensure sustained export.

Since meat is a carrier of microbiological hazards, controlling authorities of importing countries, according to Sanitary and Phyto-Sanitory agreement (SPS) of the World Trade Organizations (WTO, 2005), can ban the importation of meat, which is not microbiologically safe. However the denial should be based on risk assessment and scientific evidence. International Food Safety Committee recommend member countries to set food safety objectives and to implement Hazard

Analysis Critical Control Point (HACCP) in food processing plants (ICMSF, 2000), while this guarantees the production of safe meat. Loss of control over critical control point endangered the public health and ends up in spoiled meat .The efficacy of control measures applied is verified by conducting microbiological risk assessment in the production line (Childers and Walsh, 1992; Hathaway and Bullians, 1992). The effectiveness of the introduced control measures at each critical control point in the slaughter line is verified by testing the microbial status of meat at different stages of meat production (ICMSF, 2000).

Ethiopia will soon be a member of the world trade organization (WTO, 2005). As a future member the country will be obliged to abide to the terms of international agreement and guidelines. Failure to comply with the guidelines will have a repercussion on Ethiopian meat industry. Hence attempts should be made to be competitive in international meat trade.

Microbiological studies on sheep and goat carcasses have been conducted in local and export abattoirs in Ethiopia (Woldemariam, 2003; Wassie, 2004), however these studies dealt only with salmonella and as such data on total microbial load (aerobic mesophilic bacteria) and coliforms count in export abattoirs is lacking.

The objectives of this study were therefore: -

1. To determine the total aerobic mesophilic bacteria (AMBC) and coliform count on goat carcasses and knife blades before and after washing.
2. To compare the microbiological load of carcasses and knives between the four selected abattoirs
3. To asses the bacterial load of water used for carcass washing
4. To determine the sensitivity of *E. coli* strain isolated from coliforms found on goat carcasses in the four abattoirs to the most commonly used antimicrobial agents

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2. LITERATURE REVIEW

2.1. Microorganisms in Fresh Meat

It is generally agreed that the internal tissues of healthy slaughter animals are free of bacteria at the time of slaughter, assuming that the animals are not in state of exhaustion. When one examines fresh meat and poultry at the retail level, varying numbers and types of organisms are particular emphasis on red meats:

- The stick knife. After being stunned and hoisted up by the hind legs, animals such as steers are exsanguinated by slitting the jugular vein with what referred to as a "stick knife." If the knife is not sterile, organisms are swept into the bloodstream, where they may be deposited throughout the carcass.
- Animal hide. Organisms from the hide are among those that enter the carcass via stick knife. Others from the hide may be deposited onto the dehaired carcass or onto freshly cut surfaces. Some hide biota becomes airborne and can contaminate dressed out carcasses.
- Gastrointestinal tract. By way of punctures, intestinal contents along with the usual heavy load of microorganisms may be deposited onto the surface of freshly dressed carcasses. Especially important in this regard is the paunch or rumen of ruminant animals, which typically contains ~ 10^{10} bacteria per gram.
- Hands of handlers. This is a source of human pathogens to freshly slaughtered meats. Even when gloves are worn, organisms from one carcass can be passed onto other carcasses.
- Containers. Meat cuts that are placed, in non-sterile containers may be expected, to become contaminated with the organisms in the containers.
- Handling and storage environment. Circulated air is not an insignificant source of organisms to the surface of all slaughtered animals.
- Lymph nodes. In the case of red meats, lymph nodes that are usually embedded in fat often contain large numbers of organisms, especially bacteria. If they are cut through or added to the portions that are ground, one may expect this biota to become prominent.

In general, the most significant of the above are non-sterile containers. When several thousand animals are slaughtered and handled in a single day in the same abattoir, there is a tendency for

the external carcass biota to become normalized among carcasses although a few days may be required. The practical effect of this is the predictability of the biota of such products at the retail level (Jay, 2000).

2.1.1. Microbial Spoilage of Fresh Red Meats

Meats are the most perishable of all important foods; Meat contains an abundance of all nutrients required for the growth of bacteria, yeast, and molds, and an adequate quantity of these constituents exists in fresh meats in available form. When spoiled meat products are examined, only a few of the many genera of bacteria, molds, or yeast are found, and in almost all cases, one or more genera are found to be characteristic of the spoilage of a given type of meat product. The presence of the more-varied biota on non-spoiled meats, then, may be taken to represent the organisms that exist in the original environment of the product in questions or contaminants picked up during processing handling, packaging, and storage (Jay, 2000).

The question arises, then, as to why only a few types predominate in spoiled meats. It is helpful to remind the intrinsic and extrinsic parameters that affect the growth of spoilage microorganisms. Fresh meats such as beef, pork, and lamb, as well as fresh poultry, seafood, and processed meats, have pH values within the growth range of most of the organisms. Nutrient and moisture contents are adequate to support the growth of all organisms. Antimicrobial constituents are not known to occur in products of the type in question. Of the extrinsic parameters, temperature of incubation stands out as being of utmost importance in controlling the types of microorganisms that develop on meats, as these products are normally held at refrigerator temperatures. Essentially all studies on the spoilage of meats, poultry, and seafood carried out over the past 40 years or so have dealt with low-temperature-stored products. Odors can be detected when the surface bacterial count is between $\log_{10}$ 7.0 and $\log_{10}$ 7.5 cm^{-2}, followed by detectable slime with surface counts usually about $\log_{10}$ 7.5 to $\log_{10}$ 8.0 cm^{-2}. This is further depicted in figure 2.1.1, which relates numbers of bacteria not only to the surface spoilage of fresh poultry but to red meats and seafoods as well (Jay, 2000).

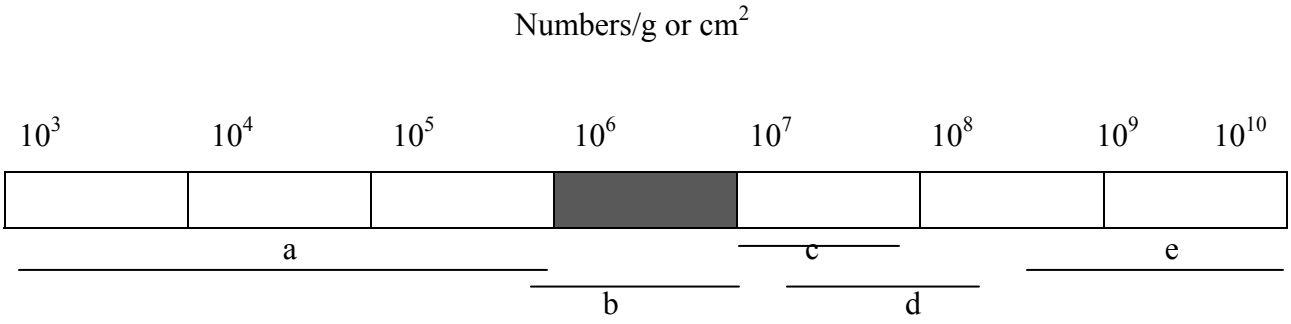


Figure 2.1.1. Significance of Total Viable Microbial Numbers in Food Products Relative to Their Use as Indicators of Spoilage.

a:- microbial spoilage generally not recognized with the possible exception of raw milk, which may sour in the 10^5 -10^6 range. b:- Some food products show incipiency in this range. Vacuum-packaged meats often display objectionable odors and may be spoiled. c:- Off-odors generally associated with aerobically stored meats and some vegetables. d:- Almost all food products display obvious signs of spoilage. Slime is common on aerobically stored meats. e:- definite structural changes in product occur at this stage.

Source: (Jay, 2000)

2.1.2. Carcass Sanitizing/Washing

Just prior to slaughter, the outer surfaces of meat animals are laden with dust, dirt, and fecal matter. It is inevitable that some of the microorganisms from these sources will be found on the carcasses of slaughtered animals, and although most are non-pathogens, pathogens may be present. In an effort to reduce the number and types of pathogens on dressed carcasses and finished products, a number of methods have emerged:

- Trimming –the excising of skin or outer tissue
- Washing- the use of plain water at varying temperatures and hose pressures

- Organic acids- the addition to wash water of acetic, citric, or lactic acid at concentrations of 2% to 5%
- Other chemicals- the addition to wash water of hydrogen peroxide, chlorine dioxide, or chlorhxidine
- Steam vacuum treatments- the application of steam for 5 to 10 seconds at 80 ^{0}C or higher as the final carcass preparation step
- Combinations- the use of two or more of the above

Overall, a large number of studies have been conducted on most of the methods noted above for removing microorganisms from slaughter carcasses, and reductions of APCs on the order of 1 to 3 log cycles is common. Many studies have employed laboratory and genetically modified strains of certain pathogens that were mixed with fresh animal feces and then rubbed on to meat cuts. The removal of biota applied in this way may be expected to be different from that acquired naturally, but comparative studies are wanting. The long-term effect of acid and steam treatments on meat biota is unknown because these procedures are relatively new for commercial use. The emergences of acid-resistant organisms after prolonged use are a likely outcome based on the long-term and widespread use of antimicrobials in general. It has been noted that multiple treatments are better than any one method alone (Jay, 2000).

2.2. Indicators of Food Microbial Quality and Safety

Indicator organisms may be employed to reflect the microbiological quality of foods relative to product shelf life or their safety from food borne pathogens (Jay, 2000).

2.2.1. Indicators of Products Quality

Microbial product quality or shelf life indictor are organisms and/ or their metabolic products whose presence in given foods at certain levels may be used to assess existing quality or, better, to predict shelf life. When used in this way, the indicator organisms should meet the following criteria (Jay, 2000).

- They should be present and detectable in all foods whose quality (or lack there of) is to be assessed.

- Their growth and numbers should have a direct negative correlation with the product quality.
- They should be easily detected and enumerated and be clearly distinguishable from other organisms.
- They should be enumerable in a short period of time, ideally within a working day.
- Their growth should not be affected adversely by other components of the food flora.

In general, the most reliable indicators of product quality tend to be product specific. When a single organism is the cause of spoilage, its numbers can be monitored by selective culturing or by a method such as impedance with the use of an appropriate selective medium. Microbial quality indicators are spoilage organisms whose increasing numbers results in loss of product quality. Metabolic products may be used to assess and predict microbial quality in some products (Jay, 2000).

Total viable count methods have been used to asses product quality. They are greater value as indicators of the existing state of given products than as predictors of shelf life because the portion of the count represented by the ultimate spoilers is difficult to ascertain.

Overall, microbial Quality indicators organisms can be used for food products that have a biota limited by processing parameters and conditions where an undesirable state of is associated consistently with a given level of specified organisms. Where product quality is significantly affected by the presence and quantity of certain metabolic products, they may be used as quality indicators. Total viable counts generally are not reliable in this regard, but they are better than direct microscopic counts (Jay, 2000).

Intrinsic in the production of quality meat fit for human consumption is the employment of competent personnel while in most countries resources have been concentrated in the past on the training of meat inspection, this emphasis in itself dose not achieve the desired results, since competent livestock handlers and meat operatives are necessary to treat animals and poultry and meat product, with efficiency and care (Gracey, 1981).

Bovine Spongiform Encephalopathy and *E. coli* 0 157 H 17 in particular, along with the introduction of some genetically modified food, have served to undermine the confidence of the consumer in the safety of food in Britain and elsewhere, the reassurance issued by authorities for the safety of food. It is difficult place reliance on often repeated pronouncements about food safety, especially when these emerge suddenly, are later contradicted, are sometimes associated

with vested interests or are suddenly imposed on the population with out evidence of adequate and competent research findings (Gracey, 1999).

The concepts of "plough to plate", "stable to table" are at last being recognized since accountability cannot be laid solely at the door of meat inspection. Livestock producers have the chief responsibility to ensure residues, are presented for slaughter with close attention being paid to welfare on the farm, through transport and plant (Gracey, 1999).

2.2.2. Indicators of Food Safety

The scientific study on food safety emerged as a discipline after the Second World War. An increasing awareness of the fact that eating quality of food commodities is determined by a logical sequence of circumstances starting at conception of the animal, or at germination of the seed, and culminating in consumption (Lawrie, 1979).

Microbial indicators are employed more often to assess food safety and sanitation than quality. Ideally, a food safety indicator should meet certain important criteria. It should: -

- Be easily and rapidly detectable
- Be easily distinguishable from other members of the biota
- Have a history of constant association with the pathogen whose presence it is to indicate
- Always be present when the pathogens of concern is present
- Be an organism whose numbers ideally should correlate with those of the pathogen concern (Figure 2.2.1)
- Posses growth requirements and a growth rate equaling those of the pathogen
- Have a die- off rate that at least parallels that of the pathogen and ideally persists slightly longer than the pathogen of concern be absent from foods that are free of the pathogen except perhaps at certain minimum numbers (Jay, 2000).

These criteria apply to most, if not all, foods that may be vehicles of food borne pathogens, regardless of their source to the foods. In the historical use of safety indicators, however, the pathogens of concern were assumed to be of intestinal origin, resulting from either direct or indirect fecal contamination. Thus, such sanitary indicators were used historically to detect fecal contamination of waters and thereby the possible presence of intestinal pathogens. The first fecal indicator was *Escherichia coli*. When the concept of fecal indicators was applied to food safety,

some additional criteria were stressed, and those suggested by Buttiaux and Mossel are still valid (Jay, 2000).

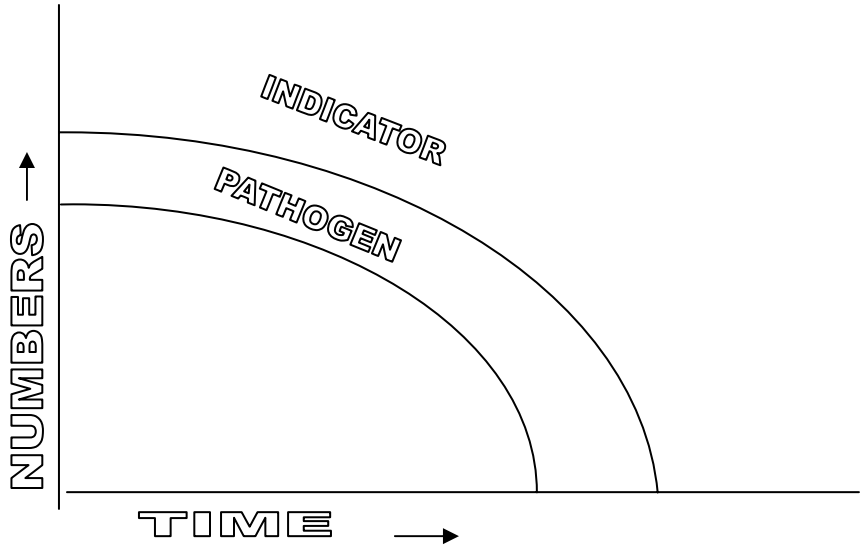


Figure 2.2.1 Idealized relationship between an indicator organism and the relevant pathogen(s).

The indicator should exist in higher numbers than the pathogen during the existence of the latter. Source: (Jay, 2000)

- Ideally the bacteria selected should demonstrate specificity, occurring only in intestinal environments.
- They should occur in very high numbers in feces so as to be encountered in high dilutions.
- They should possess a high resistance to the extraenteral environment, the pollution of which is to be assessed.
- They should permit relatively easy and fully reliable detection even when present in very low numbers.

Following the practice of employing *E.coli* as an indicator of fecal pollution of waters, other organisms were suggested for the same purpose. In time, most of these were applied to foods (Jay, 2000).

2.2.3. Coliforms

While attempting to isolate the etiologic agent of cholera in 1885, Escherich isolated and studied the organism that is now *E.coli*. It was originally named *Bacterium coli commune* because it was present in the stools of each patient he examined. Schardinger was the first to suggest the use of this organism as an index of fecal pollution because it could be isolated and identified more readily than individual waterborne pathogens. T. Smith suggested a test for this organism as a measure of drinking water potability in 1895. This marked the beginning of the use of coliforms as indicators of pathogens in water, a practice that has been extended to foods (Jay, 2000).

The presence of *E. coli* in food or water become accepted as indicator of recent fecal contaminations the possible presence of frank pathogens. Although the concept of using *E. coli* as an indirect indicator of health risk was sound, it was complicated in practice due to the presence of other enteric bacteria like *Citrobacter, Klebsiella and Enterobacter* that can also ferment lactose and are similar to *E. coli* in phenotypic characteristics, so that they are not easily distinguished. As a result, the term ”coliform” was coined to describe this group of enteric bacteria. Coliform is not a taxonomic classification, but rather a working definition used to describe a group of gram-negative, facultative anaerobic rod-shaped bacteria that ferments lactose to produce acid and gas within 48 hours at 35 ^{0}C. In 1914, the US Public Health Service adapted the enumeration of coliforms as a more convenient standard of sanitary significance (BAM, 2002).

Although coliforms were easy to detect, their association with fecal contamination was questionable because some coliforms are found naturally in environmental samples. This led to the introduction of the fecal coliforms as an indicator of fecal contamination. Fecal coliform, first detained based on the works of Eijkman is a subset of total coliforms that grows and ferments lactose at elevated incubation temperature, hence also referred to thermo tolerant coliforms. The fecal coliform group consists mostly of *E. coli*, but some other enteric microorganism such as *Klebsiella* that can also ferment lactose at this temperature and therefore, be considered as fecal coliforms. The inclusion of *Klebsiella spp*. in the working definition of fecal coliforms diminished the coalition of this group with fecal contamination. As a result, *E. coli* has remerged as an indicator, partly facilitated by the introduction of newer methods that can be rapidly identify *E. coli* (BAM, 2002).

Since *E. coli* is more indicative of fecal pollution than the other genera and species noted (especially *E. aerogenes*); it is often desirable to determine its incidence in a coliform population. The IMViC formula is the classic method used, where I = Indole production, M= Methyl red reaction, V= Voges – proskauer reaction (production of acetone) and C= Citrate utilization. By this method, the two organisms noted have the following formulas:

	I	M	V	C
E. coli	+	+	-	-
E. aerogenes	-	-	+	+

The IMViC reaction + + - - designates *E. coli* type I; *E. coli* type II strains are - + - -. The MR reaction is the most consistent for *E. coli* (Jay, 2000).

Currently; total coliforms, fecal coliforms and *E. coli* are used as indicators, but in different applications. Detection of coliforms is used as an indicator of sanitary quality of water or as a general indicator of sanitary condition in the food processing environment and *E. coli* is used to indicate recent fecal contamination or sanitary processing (BAM, 2002). The aerobic plate count (APC) is intended to indicate the level of microorganisms in a product (Maturina and Peeler, 2001).

2.3. Predictive Microbiology / Microbial Modeling

The presence/absence of indicator organisms is used to predict food safety. If a safety indicator is absent, the product is regarded as being safe relative to hazard for which the indicator is used. On the other hand, a product can have extremely low numbers of a safety indicator and yet pose a hazard. The latter is true for many food borne pathogens such as enterotoxgenic staphylococci. When low numbers of indicator or pathogen are present, it is important to know how either will behave in a food product over time. This future behavior calls into question the multiple parameters that affect the growth and activity of microorganisms in foods, and if predictions are to be made about the fate of low numbers of pathogens in a given product, how the pathogens and these parameters interact needs to be handled (Jay, 2000).

Microbial modeling or predictive microbiology is a rapidly emerging sub discipline that entails the use of mathematical models/equations to predict the growth and/or activity of a micro organism in a food product over time. The predictive or modeling aspect is not new, for it is embodied in heat-process calculations in the canning of low-acid foods. What is new is the

interest in broadening the use of this concept to a wider range of food poisoning and food spoilage organisms by the use of more sophisticated mathematical/computer models is that can handle multiple growth parameters. Predicting the growth of an organism for a single parameter is not too difficult. Difficulty arises when multiple parameters are involved, as relatively few studies have been conducted to determine their interplay on organisms. The effective application of predictive microbiology requires the selection of appropriate models to reflect the effect of growth parameters. Computer software packages for predictive microbiology are available from private and commercial sources (Jay, 2000).

2.4. General Enumeration Procedures

The examination of foods for the presence, types, and numbers of microorganisms and/or their products is basic to food microbiology. In spite of the importance of this, none of the methods in common use permits the determination of exact numbers of microorganisms in a food product. Although some methods of analysis are better than others, every method has certain inherent limitations associated its use.

The four basic methods employed for total numbers are as follows: -

1. Standard Plate Count (SPC) for viable cells
2. The Most Probable Numbers (MPN) methods a statistical determination of viable cells
3. Dye reduction techniques to estimates numbers of viable cells that posses reducing capacities
4. Direct microscopic count (DMC) for both viable and non-viable cells (Jay, 2000).

2.4.1. Standard Plate Count (SPC)/ Aerobic Plate Count (APC)

The development of agar media in the late 1800s opened the way for the development of methods for enumerating microorganisms by colony counts. These methods have been the most used procedures for determining populations of viable microorganisms. Enumeration procedures are based on the assumption that the microbial cells present in a sample mixed with an agar medium each form visible separated colonies. The Aerobic Plate Count (APC), however, does not necessarily measure the actual total numbers of viable microorganisms per gram of analyzed sample, since bacterial cells occur in pairs, chains, clusters, or clumps. The counts obtained by

these methods should not be reported as viable cell counts, but as colony counts per unit, or colony forming units per unit. When determining colony counts on a food product, it must be remembered that all types of microorganisms will not grow on a single agar medium incubated under one set of conditions. This is due to special requirements with regard to nutrients, oxygen, incubation temperature, damaged cells, or other factors. A more through representation of the total bacterial count may be obtained plating the sample on more than one nonselective medium, and incubating under more than one condition, i.e., temperature, aeration, etc. (APHA, 2001).

Colony count methodology can provide a useful tool for estimating microbial populations in foods. The optimum medium and conditions for determining the colony count may vary from one food to another. However, once the optimum procedure for a given food is determined, it can be very useful for routine microbial analysis of the food. Since minor variations in procedures can alter the results obtained with the colony count, the competency and accuracy of the analyst are very important (APHA, 2001).

2.4.1.1. Precautions and Limitations of Methods

The Aerobic Plate Count provides an estimate of the numbers viable microorganisms in foods according to the medium employed and the time and temperature of incubation. Microbial cells often occur as clumps or groups in foods. While shaking of samples and dilutions tends to uniformly distribute the clumps of bacteria, it may not completely disrupt them. Mixing the initial dilutions in a mechanical blender may provide better breakage of the clumps. However, this does not insure that the microorganisms will be distributed as a single cell in the dilutions. Consequently, each colony that appears on the agar plates can arise from a clump of cells or from a single cell, and are often referred to as colony forming units (CFU) (APHA, 2001).

The accuracy of the APC method also may be limited by the failure of some microorganism to form visible colonies on the agar medium. This failure can result from nutritional deficiencies of the medium, unfavorable oxygen tension, unfavorable incubation temperature, existence of other competing organisms (antagonistic) or cell injury. Length of incubation time, pH, water activity (a_w), oxidation reduction potential of the plating medium and temperature may also be a factor. The presence of inhibitory substances on glassware or in diluents may affect inversely some bacteria so that they will not form colonies. Other factors which may influence the accuracy of

the colony count include: improper sterilization and protection of sterilized diluents, media, and equipment, in accurate measurement of samples and dilutions, improper distribution of the sample in or the agar medium, errors in counting colonies and in computing counts. While there are some inherent limitations in enumerating microorganisms by the APC method, many of the errors can be minimized if the analyst follows directions carefully and exercises extreme care in making all measurements. Consistently accurate and meaningful results can be obtained from the routine examination of a given type of food only if each sample of that food is analyzed with the same methods or procedures. This includes sampling procedures, sample preparation of dilutions, plating medium, incubation conditions, and counting procedure (APHA, 2001).

Although the SPC is more often determined by pour plating, essentially comparable results can be obtained by surface plating. By the latter method, prepared and hardened agar plates with dry surfaces are employed. The diluted specimens are planted onto the surface of replicate plates, and, with the aid of bent glass rods ("hockey sticks"). Surface plating offers advantages in determining the numbers of heat-sensitive psychrotrophs in a food product because the organisms do not come in contact with melted agar. It is the method of choice when the colonial features of a colony are important to its presumptive identification and for most selective media. Strict aerobes are obviously favored by surface plating, but microaerophilic organisms tend to grow slower. Among the disadvantages of surface plating are the problem of spreaders (especially when the agar surface is not adequately dry prior to plating) and the crowding of colonies, which makes enumeration more difficult (Jay, 2000).

2.4.1.2. Bacterial cell counting techniques

Sometimes it is necessary in diagnostic microbiology to enumerate bacterial cells in fluids such as autogenous vaccine, water, milk or urine samples. Both viable and total counts can be carried out. Viable counting techniques are more commonly used in diagnostic and food hygiene procedures. Viable bacterial cells are capable of multiplication of visible colonies on or in agar media. In viable counting methods the assumption is made that one well-spaced, bacterial cell gives rise to one colony. Bacterial colonies, rather than the bacterial cells, are counted in most of these methods. Total counts will enumerate both viable and non-viable bacterial cells. There are inherent errors in all of these methods (Quinn, *et al*., 2002).

2.4.1.2.1. Viable counting methods

Serial ten-fold dilutions of the original fluid, containing bacterial, must first be made for each the methods. These must be carried out as accurately as possible to minimize avoidable errors and an aseptic technique should be used (Quinn, *et al*., 2002).

2.4.1.2.2. Spread plate method

A range of dilutions is used and an inoculum of 0.1 ml of each dilution is placed on the surface of an agar plate. The inoculum is spread rapidly over the entire agar surface using a thin, bent glass rod or a flame-sterilized nichrome wire, bent in a L-shape. Plate count agar, nutrient agar or even MacConkey agar can be used if a viable count of *Escherichia coli* is required. At least two and preferably four, plates should be inoculated per dilution. The plates are incubated for 24-48 hours at 25-37 ^{0}C. The incubation temperature will depend on whether environmental or pathogenic bacteria are being sought. After incubation, plates inoculated with a sample dilution yielding between 30 and 300 colonies are read, for greatest accuracy. The colony count should be an average of the two or four plates inoculated with the selected dilution. Various instruments are available to facilitate counting the colonies, including electronic counters (Quinn, *et al*., 2002).

2.4.1.2.3. Pour plate method

This method is similar to the spread-plate technique, except that the 0.1 ml inoculum is mixed thoroughly with molten agar, previously held in water bath at 50 ^{0}C. Two or four plates should be inoculated with each dilution. The agar is allowed to set and then incubated at 25-37 ^{0}C for 24-28 hours. Plates inoculated with a sample dilution that yields between 30 and 300 colonies per plate should be read. The colonies will be distributed throughout the agar as well as on the surface. The subsurface colonies assume a biconvex shape (Quinn, *et al*., 2002).

2.4.1.2.4. Miles-Misra technique

This method has the advantage of being economical with agar media. Lines can be drawn on the bottom of an agar plate with a waterproof marker, dividing it into 8 sectors. An inoculum of 0.02 ml, delivered as a drop, is placed on the agar in each sector. At least 4 drops per sample dilution should be used. The inocula are allowed to dry and the plates incubated at 25-37 ^{0}C for 24-48

hours. A sample dilution yielding about 30 colonies per drop should be selected. An average colony count from at least 4 drops must be obtained. The calculation is similar to that for the two previous methods, but as the inoculum was 0.02 ml, the conversion factor will be 50 to obtain a figure for the bacteria/ml in the original sample (Quinn, *et al*., 2002).

2.4.1.2.5. Filtration method

This is a useful method for determining the number of bacteria in a water sample or other clear fluid where the bacterial number is low. A known volume of water is passed through a membranes filter of pore size 0.22 μm. The filter will retain the bacterial cells, and is aseptically placed, bacterial-side up, on the surface of an agar plate. The medium can be selective or non-selective, depending on the bacterial species being sought. Colonies will form on the surface of the filter after incubation and can be counted. As the volume, of the water or fluid is known, the bacteria/ml or per 100 ml, of samples can be calculated (Quinn, *et al*., 2002).

2.4.1.3. Total counts of bacterial cells

These methods do not distinguish between viable and non-viable cells and thus the bacterial count will include both living and dead cells (Quinn, *et al*., 2002).

2.4.1.3.1. Breed's direct smear method

This technique is used most commonly for counting bacteria in milk. A grease-free microscope slide is placed over a template 1 cm x1cm (area of 100 mm^2) and a 0.01 ml of sample is carefully spread over this area. The smear is allowed to air-dry, fixed by heat and stained with methylene blue for about 1 minute. After air-drying, the stained smear is examined under the oil-immersion objective. The bacterial cells should be counted in at least 50 fields throughout the area of the smear. An average bacterial cell count per field (N) should be obtained. The radius (r) for the particular microscope's oil-immersion field can be found (in mm) using a slide and eyepiece micrometer. The area of the field will be πr^2 or approximately 3.14x r^2 (mm^2) (Quinn, *et al*., 2002).

2.4.1.3. 2. Counting chamber method

The Helber chamber was specially developed for counting unstained bacteria in a suspension and is supplied by Hawksely Ltd, London, but is not always readily available. An improved Neubauer haemocytometer can be used instead. The technique for counting bacterial cells and the calculation of bacteria/ml in the liquid sample is similar to that for erythrocytes. To prevent motility of the bacteria, 2-3 drops of full strength formalin/10 ml of bacterial suspension can be added. The chamber is filled and viewed under the low-power objective in order to orientate the marked grid. Then the high-dry objective is used to count the bacteria in the five areas in the central region of the grid. Each of these large squares, one at each corner and one central, is divided into 16 smaller squares. Thus, the bacterial cells are counted in 80 (5x16) of the smallest squares in the grid. The average number of bacteria per small square can be calculated (Quinn, *et al*., 2002).

2.4.1.3.3. Surface contact plates

Special plastic plates are available to allow direct sampling of flat surfaces for bacteria. The technique can be used to detect a specific pathogen, such as salmonella, when a selective medium would be appropriate, or to determine the degree of contamination of a surface using a non-selective medium. An exact quantity of agar must be used to fill these plates, as the agar surface should project slightly above the rim of the plate. Surfaces are sampled by placing the agar gently on the area, the plate lifted carefully and the lid replaced. The plates are incubated at 30-37 ^{0}C for 24-48 hours and examined for colonial growth. If required the number of bacteria/cm^{2} of surface could be calculated, as the plastic plates incorporate a grid on the base (Quinn, *et al*., 2002).

2.4.2. Most probable numbers

In this method, dilutions of food samples are prepared as for the SPC. Three serial aliquots or dilutions are then planted into 9 or 15 tubes of appropriate medium for the three-or five-tube method, respectively. Numbers of organisms in the original sample are determined by use of standard MPN tables. The method is statistical in nature, and MPN results are generally higher than SPC results. McCrady introduced this method in 1915. It is not a precise method of analysis;

the 95% confidence intervals for a three-tube test range from 21 to 395. When the three-tube test is used, 20 of the 62 possible test combinations account for 99% of all results, whereas with the five-tube test, 49 of the possible 214 combinations account for 99% of all results. In a collaborative study on coliform densities in foods, a three-tube MPN value of 10 was found to be as high as 34, whereas in another phase of the study, the upper limit could be as high as 60. Although Woodward concluded that many MPN values are improbable, this method of analysis has gained popularity. Among the advantages it offers are the following:

- It is relatively simple.
- Results from one laboratory are more likely than SPC results to agree with those from another laboratory.
- Specific groups of organisms can be determined by use of appropriate selective and differential media.
- It is the method of choice for determining fecal coliform densities.

Among the drawbacks to its use are the large volume of glassware required (especially for the five-tube method), the lack of opportunity to observe the colonial morphology of the organisms, and its lack of precision (Jay, 2000).

These techniques are based on statistical probabilities with the assumption that there is a normal distribution of bacteria in liquid samples. If the liquid sample contains one viable bacterial cell, its growth and multiplication in a suitable broth can be detected by manifestations such as turbidity or acid and gas production. The methods can be used for the detection of coliform bacteria in water supplies. MacConkey broth with bromocresol purple as the pH indicator is often used for coliform counts. Acid production is indicated by a yellow colouration of the broth and gas is trapped by a Durham tube. One recommended method is to take one 50 ml, five 10 ml and five 1 ml quantities of the water sample. The 50 ml and 10 ml volumes are each added to their own volume of double-strength broth, while the 1 ml samples are each added to 5 ml of single-strength broth. The inoculated tubes are incubated at 35 ^{0}C for 48 hours and then each is examined for acid and gas production. By referring to standard MPN probability tables the MPN of coliforms/100 ml of water sample can be determined. For example, if one each of the tubes inoculated with 50 ml, 10 ml and 1 ml samples of water, respectively, showed acid and gas production, then from the tables the MPN of coliforms/100 ml water would be 5. For the differential coliform count specifically to detect *Escherihia coli*, tubes showing acid and gas

production are subcultured into fresh MacConkey broth and incubated at 44 ^{0}C. Formation of acid and gas within 48 hours at this temperature is presumptive for *E. coli* and indicative of fecal pollution of the water (Quinn, *et al*., 2002).

2.4.3. Turbidity standards

Brown's or McFarland's opacity tubes are available commercially. They consist of a series of ten numbered, standard, thin glass tubes containing different dilutions of suspended barium chloride or barium sulphate, giving a range of opacities. The test bacterial suspension is placed in a 'blank' tube of similar dimensions to the standards. Rolling the test suspension across a printed page and matching it with a standard of comparable opacity make a visual comparison of opacity. Tables are supplied with the opacity tubes that give the numerical equivalents (bacteria/ml) of the opacity standards for a certain range of bacteria. It is a convenient and simple method, but gives only an approximate total bacterial count (Quinn, *et al*., 2002).

2.4.4. Coulter counter

Coulter counters are automated, electronic counting instruments, usually used in hematology, but they can be adjusted to conduct total bacterial cell counts (Quinn, *et al*., 2002).

2.5. Surface Contamination of Carcasses by Fecal Coliforms

The meat inspectors today are mainly looking for visible pathological findings such as arthritis and pleuritis, but invisible hazardous elements such as pathogenic bacteria have been receiving more attention during recent years (Hansson, 2000).

When animals are presented for slaughter they have a large and very variable population of aerobic microorganisms on their hooves, hides and within their intestinal tract. An absolute guarantee can never be given that the alimentary tract of healthy animals will be free from micro organisms pathogenic to humans (Huis in'l, *et al*., 1997).

Generally the internal surfaces of the carcasses are sterile, and transfer of bacteria results from dressing and skinning defects occurring during the slaughter process (Gill and Penny, 1979).

Minimizing bacterial contamination of carcasses during and immediately after slaughter improves the microbiological safety of meat. Most bacterial contamination of carcass surfaces occurs during slaughter and/or dressing procedures from a variety of sources, such as hides,

intestinal contents, contact surfaces, and handling by workers (Hansson, *et al*., 1990). Contaminated hide during transport can be reduced if feed is withheld for 3 to 6 hours before trucking (Mackey and Roberts, 1990).

If feed is not withdrawn before slaughter, the intestinal tract contains a large amount of ingesta, it is more difficult to handle during evisceration, and it is more likely to break or be accidentally cut, causing carcass contamination. To avoid contamination during removal of the skin, it is essential that the hide does not touch the already dehided surfaces. The great majority of mesophilic or psychotrophic bacteria found on cattle carcasses immediately after slaughter and dressing derive from hide (Newton, *et al*., 1978). Wet and dirty hides from cattle may be the source of a strong contamination of the dehided carcasses (Snijders, *et al*., 1984). Excessive moisture conditions generally results in higher levels of hide contamination (NACMSF, 1993).

It is generally accepted that the use of Good Manufacturing Practices (GMP) at the slaughter house will correlate with low carcass bacterial counts. However, even under GMP's some bacterial carcass contamination inherent to slaughtering can be expected. On the other hand, contamination is primarily uneven because of accidental contact with contaminated materials. Thus, sampling small areas will be in appropriate to determine differences between abattoirs (Lasta and Fonrough, 1998). Other factors, such as visit to the abattoir for sample- taking and season when samples are taken, might affect carcass counts (Lasta, *et al*., 1992).

To estimate bacterial counts on meat surfaces, many variables should be taken in to account, such as microbiological technique, differences in sampling sites, types of carcass, time of the year, day and hour of sampling, differences between establishments (Stolle, 1988). Another factor of paramount importance is the choice of the indicator organism. There is no reason to expect the protection of public health to evolve from bacteriological standards, which limit the number of nonpathogenic organisms (Gopfert and Kim, 1975). Nevertheless, the total plate count, the enterobacteriaceae count, and the fecal coliform count have an indicator function for processing hygiene and storage quality (Nortje, *et al*., 1990).

The initial microbial load of the carcass is mainly determined by surface contamination acquired during slaughtering, dressing, chilling and cutting. The almost sterile subcutaneous muscle tissue at the moment of hide removal becomes contaminated within a few tenths of a second to a level of 10^4 bacteria / cm^2 (Kiraa, *et al*., 1985). Even brief contact with fecal material can produce

contamination of up to 10^6 bacteria/ cm^2; enough to cross contaminate 10 or more successive carcass (Roberts, 1980).

During cutting and boning operations, the major source of contamination is likely to be the surface of the incoming carcasses combined with the degree to which this contamination is spread to freshly cut surfaces by contact with meat surfaces, equipment, cutting boards or tool (ICMSF, 1980).

Since *E. coli* frequently represents the majority of fecal coliform count, reducing the latter on boneless beef could also assist in minimizing the risk of *E. coli* infection. The cutting and boning operations are the most significant contributors to increased fecal coliform counts on boneless beef. Therefore, it is crucial to exert controls not only at the slaughter house level to minimize carcass contamination during dressing operations but also, and especially, at the boning room level to reduce the cross- contamination created by meat handlers and direct contact surfaces (Charlebois, *et al*., 1991).

2.6. Attachment and Proliferation of Bacteria on Meat

Microbial contamination of raw meat has always been an important issue for food safety. One measure to ensure good meat quality is to rely on effective washing of carcasses in order to decrease the microbiological population on the surfaces of the meat (Crouse, *et al*., 1988 and Kotula, *et al*., 1974). Although methods and devices have been developed to clean carcass surfaces (Anderson, *et al*.,1982; Anderson, *et al*., 1982 and Anderson, *et al*., 1983), complete sterilization of raw meat is difficult to achieve. To ascertain if washing succeeds, basic information on microbial attachment to meats is essential. Although rates of bacterial attachment to meat, especially chicken, have been studied, (Lillard, 1985; Notemans and Kampelmacher, 1974), there is a limited information on the bacterial attachment to fat tissue in comparison to the lean muscle tissue of red meat. Fat tissue may provide a better surface than lean muscle tissue for bacterial attachment since their surface structures are entirely different. Microbial spoilage of meat is influenced not only by their initial attachment to surface, but also by subsequent proliferation after attachment. When meat is inoculated with bacteria, bacterial growth took place. Lean muscle supported bacterial proliferation better than fat tissue for all bacteria tested except *P. aeruginosa. P. aeruginosa* grow much better in fat tissue than in the lean meat (Chung, 1989). Although fat tissue is pliable and hydrophobic, there were basically no differences in

attachment between fat and lean tissue. Bacteria attached to the fat tissue may be more difficult to wash off, because fat tissue is hydrophobic (Chung, 1989).

2.7. Microbiological Contamination of Ovine Carcasses Associated with the Presence of Wool and Fecal Material

The presence of fecal material or wool on the carcass was not associated with increased bacterial number on visually clean areas of the carcass. This indicates that the presence of fecal material or wool alone cannot be used as indicator of the hygienic status of the carcass as a whole, particularly in the role of on line monitoring parameters for HACCP systems. Pre-evisceration washing of the carcasses had very little effect on the uncontaminated areas of the carcasses, but reduced the mean Aerobic plate count (APC) and *Escherchia Coli* count (EC) at the site of visible contaminants. There was little evidence of redistribution of bacteria to immediately adjacent but visually clean sites. However, the residual levels of both APC and ECs directly at sites of fecal contamination after washing were still significantly higher than at visually clean sites. Application of HACCP principles to ovine slaughter and dressing suggests that visible fecal material should be removed by trimming, where as pre-evisceration washes can have practical and microbiologically validated role in the removal of wool. There was generally a good correlation between APCs and ECs at the uncontaminated sites prior to pre-evisceration washing, suggesting that in some situations APCs can act as a useful indicator of both general carcass hygiene and the presence of fecal indicator (Biss and Hathaway, 1995).

The hide and gastrointestinal tract represent the most significant Sources of bacterial contamination for red meat carcasses (Bell and Hathaway, 1996). The viscera are generally removed from the carcass intact and direct contamination of carcasses only occurs if the gastrointestinal tract is ruptured. However, fecal material may be present on the hide or fleece, and its careless removal can result in the transfer of bacteria to the carcass surface (Nottingham, *et al*., 1974; Roberts, 1980; Eustace, 1981). Additional carcass contamination can arise from aerosols generated during slaughter and dressing process (Biss and Hathaway, 1994a, 1996).

The presence of visible wool, dirt and fecal material on the ovine carcasses is commonly used as an indicator that dressing hygiene is inadequately controlled and this is associated with an

increased potential for food borne disease and product spoilage. According to traditional practice, regulatory controls and penalties may be imposed on the processor if the presence of visible contaminants, especially fecal material exceeds pre-determined limits (Anon, 1991a, 1993a and 1993b).

HACCP systems for red meat process control are increasingly being considered more appropriate than traditional end point inspection systems for the control of public health hazards associated with red meat (Hathaway and Macknzie, 1991; Hague, *et al*., 1993; Anon, 1994; Bullians and Hathaway, 1994; Biss and Hathaway, 1995). HACCP Depends upon on line monitoring so that immediate corrective action can be taken if the process is not controlled with in specified limits. It is assumed that in the absence of rapid on line microbiological tests, generic HACCP models for slaughter and dressing hygiene will be serviced by monitoring visible contamination (particularly that which is gastrointestinal in origin). This trend has developed despite the contention that inspection for visible dirt on carcasses does not ensure microbiological cleanliness (Mackey and Roberts, 1993) and that meat borne pathogens cannot be detected by visual examination (Smulders and Van Laack, 1992).

In fact, recent work has demonstrated that pre-slaughter washing of wooly lambs decrease visible contamination on carcasses but actually increase levels of microbiological contamination (Biss and Hathaway, 1996).

Pre-evisceration washing of sheep carcasses and post-evisceration washing of beef carcasses have been used to remove visible contamination (Roberts, 1980; Anderson, *et al*., 1991; Biss and Hathaway 1994b). Numerous studies have demonstrated however that there is only limited value in microbiological terms unless a sanitizing agent such as chlorine, acetic or lactic acids, or heat is also used (Kotula, *et al*., 1974; Eustace, 1981; Kelly, *et al*., 1982; Dickson and Anderson, 1992; Ellerboek, *et al*., 1993). However, it is generally considered highly undesirable that carcasses visibly contaminated with fecal materials proceed through the wash without prior trimming, since it is assumed that washing will disseminate bacteria over other parts of the carcass (Anon, 1991a, 1994).

Design and Validation of genuine HACCP plan for red meat slaughter and dressing requires microbiological validation of the critical control points (CCPs) that are identified and the limits that are identified and the limits that are set to meet the objectives of the HACCP plan (Hathaway, 1994).

The level of visible contamination of the carcasses is commonly used as an indicator of the hygienic standard of slaughter and dressing process in HACCP plans developed for red meat slaughter and dressing systems (Anon, 1994, 1995b).

Pre-evisceration washes have been used for many years in many countries to remove inadvertent contamination with wool, dust and blood and to improve the final appearance of the carcasses. Fecal contamination may be present, and a persistent criticism of pre-evisceration washing is that potential pathogens associated with fecal material could be redistributed over the carcass (Anon, 1993c).

It has been found that the mean *E. coli* counts mirrored the directional trends of the mean APCs in response to various process interventions understudy. It appears therefore, that increasing mean APCs will be a reasonable indicator of increasing mean *E. coli* counts on the carcasses, and by implication increasing levels of potential human pathogens of gastrointestinal origin. However, this relationships is most evident immediately after pelting and before a general homogenization of the bacterial populations is effected by further process interventions such as carcass washing (Biss and Hathaway, 1996).

Careful handling at the different stages of processing of sheep reduced the level of microbial contamination of the carcasses processing steps such as evisceration and washing did not increase the microbial counts on the carcass surface. The major sources of microbial contamination are skin, floor washings, intestinal contents and gambrels; seasonality did not have any effect on the microbial contamination of carcasses (Rao and Rammesh, 1992).

Many changes have occurred in the handling, processing, packaging, preservation and distribution of meat products in relation to microbiology. Microbial contamination results in spoilage of meat, reduced shelf life of meat and public health hazards. Therefore, it has become of major concern to study the microbiology of meat in order to determine potential safety and keeping quality. Changes in life styles have made consumers more aware of the quality of meat. Meat eaters have become more selective and critical and conscious of quality, particularly freshness, wholesomeness and palatability (Nottingham, 1982).

Effectiveness of washing varies with the time spent on washing, and volume, pressure and temperature of the water and design of the spraying device (Baily, 1971).

The bacterial counts of the carcass after skinning, after evisceration and after washing did not differ significantly with the careful handling of carcasses during skinning, evisceration and

washing. Skin, floor washing, intestinal contents and gambrels carried high microbial population and were the major source of contamination. However, knives, walls, workers' hands, trolleys and gum boots were found to carry large numbers of micro-organisms and were also important source of microbial contamination (Rao and Ramesh, 1992).

In the last decade, infections caused by verocytotoxigenic *E.coli* O 157 (VTEC O157) and other VTEC have emerged as a major public health concern in North America and in Europe. This anxiety stems both from the increased numbers of infections, which have been reported, and the wide spectrum of illness, which may ensue, ranging from mild diarrhea through to hemorrhagic colits, Haemolitic Uramic Syndrome (HUS) and Thrombtic Thrombocytopaennic Purpura (TTP) (Coia, 1998). The technique of Immunomgnetic separation (IMS) has revolutionized the ability to isolate the VTEC O157, with an increase in sensitivity of between10 and 100 fold (Wright, *et al*., 1994).

HACCP is generally accepted to be the most effective means of preventing microbial contamination of meat carcasses during slaughter (Bolton, *et al*., 2002). Thus recent (2001/471/EC) and future (hygiene regulations) EC legislation mandates HACCP in all meat and poultry plants throughout the European Union. Further more, these new laws set out microbial performance standards, based on excision sampling, which must be achieved. Excision was chosen as the most appropriate carcass sampling technique because several studies suggests that the excision procedure achieves a higher recovery of bacteria from meat carcasses as compared to other methods such as swabbing (Palumbo, 1999; Rivas, 1995 and Sharpe, 1986). However, this is not a consensus opinion and other research would suggest that with abrasive materials may be as effective as excision (Dorsa, *et al*., 1996;Gill and Jones, 2000;Ware, *et al*., 1999). Further more, excision is destructive, laborious and often difficult to apply in commercial circumstances and is therefore not the method of choice of industry personnel (Dorsa, *et al*., 1996; Dorsa, *et al*., 1997; Gill, *et al*., 2001).

2.8. Comparison between Destructive and Non Destructive Sample Taking Methodology

The destructive method and non-destructive method are the two methods to collect samples from carcasses. The destructive method includes cork borer method and template excision method. The

non-destructive method includes; wet and dry swab method, sponge sampling method and gauze tampon method (ISO 17604, 2003).

The excision of surfaces tissue harvests all bacteria on the surfaces while other methods do not. This invariably results in larger counts by the destructive method. Not all bacteria on the surface may be removed by either methods or grow on the media and incubation conditions used.

Repeatability and reproducibility of destructive methods are less variable, because the sampling methods used in non-destructive testing result in greater operator variability. Only a small proportion of the carcass is sampled by destructive methods, which may result in significant in accuracies when total contamination is low and heterogeneously distributed, or when the presence of the target pathogen sparse. Excision caused damage to the carcass, which may be commercially unacceptable (ISO 17604, 2003).

2.8.1. Advantages of destructive methods

The excision of surface tissue harvests all bacteria on the surface while other methods do not. This invariably results in larger counts by the destructive method. Not all bacteria on the surface may be removed by either method or grow on the media and incubation conditions used. Repeatability and reproducibility of destructive methods are less variable, because the sampling methods used in non-destructive testing result in greater operator variability (ISO 17604, 2003).

2.8.2. Disadvantages of destructive method

Only a small proportion of the carcass is sampled by destructive methods, which may result in significant inaccuracies when total contamination is law and heterogeneously distributed, or when the presence of the target pathogen is sparse. Excision causes damage to the carcass, which may be commercially unacceptable (ISO 17604, 2003).

2.8.3. Calculations of the diagnostic value

Swabbing of pork carcasses recovers on average only 30% of the number of enterobacteriaceae that can be recovered by sampling a comparable surface area with the destructive method. Also the reproducibility of swabbing is poor and large variations in results are therefore common.

Detecting beef carcasses contaminated with *Escherichia coli* or coliform microorganisms the sensitivity of swabbing a surface of 100 cm^2 is only 30% to 40% when compared to excising 100

cm^2. The calculated Kappa-value of 0.22 means that there is rather poor agreement between the results of both methods (Table 2.8.1). Furthermore, Table 2.8.2 demonstrates that at low levels of contamination even the destructive method is in practice not as consistently robust as might be expected. Table 2.8.3 presents an approximation of the absolute sensitivity of swabbing and the destructive method. The estimated sensitivity of about 80% for the destructive method (11/14), is always better than the estimated sensitivity of about 50% for swabbing (7/14), but both techniques lead to an under-estimation of the true prevalence (ISO 17604, 2003).

However, exact knowledge of the diagnostic value (i.e. the sensitivity, the specificity, the precision and the predictive value) of the still widely used (classical) sampling methods is not available in the literature (ISO 17604, 2003).

2.8.4. Sampling points

Sampling in the chill room 12 h to 24 h after slaughter may not be appropriate in all cases. Where flash chilling of pig carcasses at, for example, –30 ^{0}C to 35 ^{0}C is used, many pathogens are likely to be killed or sub lethally injured, and hardening of the fat tissue makes it more difficult to recover bacteria (ISO 17604, 2003).

Table 2.8.1. Swabbing Compared with Excision Regarding the Detection of Carcasses with about 16 CFU Coliforms Per Square Centimeters

		Detected with excision (100 cm^2)		
		Yes	No	Total
Detected with swabbing (100 Cm^2)	Yes	4	3	7
	No	7	16	23
	Total	11	19	30
Relative sensitivity (4/11)		36%		
Relative specificity (16/19)		84%		
Relative predictive value positive result (4/7)		57%		
Relative predictive value negative result (16/23)		69%		
Relative precision (14+16)/30		67%		
Apparent prevalence (7/30)		23%		
True prevalence [a] (11/30)		37%		
Observed agreement between methods (20/30)		0.666		
Positive agreement (yes/yes) by chance (7/30) x (11/30)		0.086		
Negative agreement (no/no) by chance (23/30) x (19x/30)		0.486		
Total agreement by chance (a)		0.572		
Observed agreement minus total agreement by chance (b)		0.094		
Maximum agreement outside of chance (1-a)		0.428		
Cohens' kappa [b] [bl(1-a)]		0.220		

Note Calculated with data from reference (4)

[a] True prevalence as determined with excision

[b] A kappa value of between 0.4 and 0.7 is usual and represents fair to good agreement. A kappa value of 0.22 represents poor agreement

Source: (ISO 17604, 2003)

Table 2.8.2. Diagnostic Value of Swabbing and Excision with Regard to the Detection of Carcasses with about 16 CFU Coliform Organisms Per Square Centimeters

	Are about 16 CFU coliforms/ cm^2 actually present on carcasses?			
		Yes	No	Total
Detected with excision (100 cm^2)	Yes	11	0	11
	No	3	16	19
	Total	14	16	30
Detected with swabbing (100 cm^2)	Yes	7	0	7
	No	7	16	23
	Total	14	16	30

Source: (ISO 17604, 2003)

Table 2.8.3. Evaluation of Swabbing and Excision Methods

Method evaluation [a]	Excision	Swabbing
Sensitivity	79%	50%
Specificity	100%	100%
Positive predictive value	100%	100%
Negative predictive value	84%	70%
Precision	90%	77%
Apparent prevalence	37%	23%
True prevalence	47%	47%
[a] See Table 2.8.1. for calculation of sensitivity, specificity, predictive value, precision, etc		

Source: (ISO 17604, 2003)

Excision and swabs Total Viable Counts (TVCs) were statistically similar ($P<0.05$) at all sites. The corresponding Total Enterobacteriaceae counts (TECs) were also statistically the same ($P< 0.05$). Swabbing with polyurethane Sponge was therefore as effective as excision sampling

for the determination of TVCs and TECs on bovine and ovine carcasses and may be used instead of excision sampling when assessing bovine and ovine carcasses hygiene as peer 2001/471/EC (Byrne, *et al*., 2005). Article 2 of 2001/471/EC permits the use of procedures other than excision to be used if it is demonstrated that these are at least equivalent (Anon, 2001).

Excision is an effective technique for bacterial recovery from meat carcasses (Bolton, 2003). Swabbing with a sufficiently abrasive material may be as effective as excision for bacterial recovery from carcasses. Cotton wool swabbing is the least effective technique, excision yields highest bacterial recovery and in between there are a variety of sponges, clothes and meshes which range inefficiency depending on their abrasiveness (Byrne, *et al*., 2005).

In contrast to highly processed foods, most foods of animal origin passes a relatively low level of quality confidence. This is mainly due to the complexity and the length of the meat production chain and the lack of absolute control over critical points in the over all production of and processing system. Generally speaking, the better the quality of a product can be maintained by longitudinally integrated safety assurance, the less need there is for extensive microbiological monitoring. However, it must be realized that the majority of critical points in meat production are classified as CCP2, critical points which cannot be absolutely controlled (ICMSF, 1988).

2.9. The HACCP System and Food Safety

Among the desirable qualities that should be associated with foods is freedom from infectious organisms. Although it may not be possible to achieve a zero tolerance for all such organisms under good manufacturing practices (GMP), the production of foods with the lowest possible numbers is the desirable goal. With fewer processors producing more products that lead to foods being held longer and shipped farther before they reach consumers, new approaches are needed to ensure safe products. Classic approaches to microbiological quality control have relied heavily on microbiological determinations of both raw materials and end products, but the time required for results is too long for many products. The development and use of certain rapid methods have been of value, but these alone have not obviated the need for newer approaches to ensuring safe foods. The hazard analysis critical control point (HACCP) system is presented as the method of choice for ensuring the safety of foods from farm to table. When deemed necessary,

microbiological criteria may be established for some ingredients and foods, and these in connection with sampling plans are presented as components of the HACCP system (Jay, 2000).

2.9.1. Hazard analysis critical control point system

HACCP is a system that should lead to the production of microbiologically safe foods by analyzing for the hazards of raw materials –those that may appear throughout processing and those that may occur from consumer abuse. It is a proactive, systematic approach to controlling food borne hazards. Although some classic approaches to food safety rely heavily on end product testing, the HACCP system places emphasis on the quality of all ingredients and all process steps on the premise that safe products will result if these are properly controlled. The system is thus designed to control organisms at the point of production and preparation. Because the application of the HACCP system provides for the most specific and critical approach to the control of microbiological hazards presented by foods, use of this system should be required of industry. Accordingly, this subcommittee believes that government agencies responsible for control of microbiological hazards in foods should promulgate appropriate regulations that would require industry to utilize the HACCP system in their food protection programs. Before an HACCP program is developed, there are some prerequisite programs that should be in place (Jay, 2000).

2.9.2. Prerequisite programs

Prerequisite programs include a wide range of activities and events that may have an impact on an HACCP system for a specific food product even though they are not parts of the HACCP system per se. Briefly stated prerequisite programs include concerns and aspects of the entire food environment before the HACCP system are initiated. They include the suitability of facilities, control of supplier's safety and maintenance of production equipment, cleaning and sanitation of equipment and facilities, personal hygiene of employees, control of chemicals, pest control, and the like. These prerequisites include good manufacturing practices, and they should be brought up to acceptable standards before the HACCP system is initiated (Jay, 2000).

2.9.3. Definitions.

The following terms and concepts are valuable in the development and execution of an HACCP system and are taken from International Commission on Microbiological Specifications for Foods (ICMF) and/or National Advisory Committee on the Microbiological Criteria for Foods (NACMCF).

Control point: Any point in a specific food system where loss of control does not lead to an unacceptable health risk.

Critical control point (CCP): Any point or procedure in a food system where control can be exercised and a hazard can be minimized or prevented

Critical limit: One or more prescribed tolerances that must be met to ensure that a CCP effectively controls a microbiological health hazard

CCP decision tree: A sequence of questions to assist in determining whether a control point is a CCP.

Corrective action: Procedures followed when a deviation occurs

Deviation: Failure to meet a required critical limit for a CCP

HACCP plan: The written document that delineates the formal procedures to be followed in accordance with these general principles

Hazard: Any biological, chemical, or physical property that may cause an unacceptable consumer health risk (unacceptable contamination, toxin levels, growth, and / or survival of undesirable organisms)

Monitoring: A planned sequence of observations or measurements of critical limits designed to produce an accurate record and intended to ensure that the critical limit maintains product safety

Risk category: One of six categories prioritizing risk based on food hazards

Validation: That element of verification focused on collecting and evaluating scientific and technical information to determine whether the HACCP plan, when properly implemented, will effectively control the hazards

Verification: Methods, procedures, and tests used to determine whether the HACCP system is in compliance with the HACCP plan (Jay, 2000).

2.9.4. HACCP principles

Although interpreted variously, the ICMF and NACMCF view HACCP as a natural and systematic approach to food safety and as consisting of the following seven principles:

1. Assess the hazards and risks associated with the growing, harvesting, raw materials, ingredients, processing, manufacturing, distribution, marketing, preparation, and consumption of the food in question.
2. Determine the CCP(s) required controlling the identified hazards.
3. Establish the critical limits that must be met at each identified CCP.
4. Establish corrective actions to monitor the CCP(s)
5. Establish corrective actions to be taken when there is a deviation identified by monitoring a given CCP.
6. Establish procedures for verification that the HACCP system is working correctly.
7. Establish effective record keeping systems that documents the HACCP plan (Jay, 2000).

2.9.4.1. Principle 1: Assess Hazards and risks

Hazards and risks may be assessed for individual food ingredients from the flow diagram or by ranking the finished food product by assigning to it a hazard rating from A through F. A plus sign (+) is assigned when a hazard exists. Six hazards categories have been defined, representing an expansion of the three proposed by the National Research Council (NRC) for *Salmonellae* control. However, this system of ranking and hazard category assignment is not popular in the late 1990s and it may be ignored. It is presented here for historical purposes:

A. This is a special class of foods that consist of non-sterile products designated and intended for consumption by individuals at risk, including infants, the aged, infirmed, and immunoincompetents.
B. The product contains “sensitive” ingredients relative to microbiological hazards (e.g., milk fresh meats).
C. There is no controlled processing step (such as heat pasteurization) that effectively destroys harmful microorganisms.

D. The product is subject to recontamination after processing but before packaging (e.g. pasteurized in bulk and then packaged separately).

E. Substantial potential for abusive handling exists in distribution and/or by consumers that could render the product harmful when consumed (e.g. products to be refrigerated are held above refrigerator temperatures).

F. There is no terminal heat process after packaging or when cooked in the home.

Next, the formulated product should be assigned to one six hazard categories, expanded from four suggested by the NRC.

VI. A special category that applies to non-sterile products designated and intended for individuals in hazard category A

V Food products subject to all five general hazard characteristics (B, C, D, E &F)

IV Food products subject to any four general hazard characteristics

III Products subject to any three of the general hazard characteristics

II Products subject to any two general hazard characteristics

I Products subject to any one of the general hazard characteristics

0 Products subject to no hazards (Jay, 2000).

2.9.4.2. Principle 2: Determine CCP(s)

The ICMF recognized two types of CCPs: CCP1, to ensure control of hazard, and CCP2, to minimize a hazard. Typical of CCPs are the following:

- Heat process steps where time-temperature relations must be maintained to destroy given pathogens
- Freezing and time to freezing before pathogens can multiply
- The maintenance of pH of a food product at a level that prevents growth of pathogens
- Employee hygiene (Jay, 2000).

2.9.4.3. Principle 3: Establish Critical Limits

A critical limit is one or more prescribed tolerances that must be met to ensure that a CCP effectively controls a microbiological hazard. This could mean keeping refrigeration's

temperatures within a certain specific and narrow range or making sure that a certain minimum destructive temperature is achieved and maintained long enough to effect pathogen destruction (Jay, 2000).

2.9.4.4. Principle 4: Establish Procedures To Monitor CCPs

The monitoring of a CCP involves the scheduled testing or observation of a CCP and its limits; monitoring results must be documented. If for example, the temperature for a certain process step should not exceed 40 ^{0}C a chart recorder may be installed. Microbial analyses are not used to monitor since too much time is required to obtain results, Physical and chemical parameters such as time, pH, temperature, and water activity (a_w) can be quickly determined and results obtained immediately (Jay, 2000).

2.9.4.5. Principle 5: Establish Corrective Actions

Establish corrective actions to be taken when deviations occur in CCP monitoring. The action taken must eliminate the hazard that was created by deviation from the plan. If a product is involved that may be unsafe as a result of the deviation, it must be removed. Although the actions taken may vary widely, in general they must be shown to bring the CCP under control (Jay, 2000).

2.9.4.6. Principle 6: Establish Procedures for Verification

Establish Procedures for verification that the HACCP system is working correctly. Verification consists of methods, procedures, and tests used to determine that the system is in compliance with the plan. Verification confirms that all hazards were identified in the HACCP plan when it was developed, and verification measures may include compliance with a set of established microbiological criteria when established. Verification activities include the establishment of verification inspection schedules, including review of the HACCP plan, CCP records, deviations, random sample collection and analysis, and written records of verification inspections. Verification inspection reports should include the designation of persons responsible for administering and updating the HACCP plan, direct monitoring of CCP data while in operation,

certification that monitoring equipment is properly calibrated, and deviation procedures employed (Jay, 2000).

2.9.4.7. Principle 7: Establish Effective Record keeping System

Establish effective record keeping systems to document the HACCP plan. The HACCP plan must be on file at the food establishment and must be made available to official inspectors upon request. Forms for recording and documenting the system may be developed, or standard forms may be used with necessary modifications. Typically, these may be forms that are completed on a regular basis and filed away. The forms should provide documentation for all ingredients, processing steps, packing, storage and distribution (Jay, 2000).

2.9.5. Some Limitations of HACCP

Although it is the best system yet devised for controlling microbial hazards in foods from the farm to the table, the uniform application of HACCP in the food manufacturing and service industries will not be without some debate. Among the lingering questions and concerns raised by Tompkin are the following:

- HACCP requires the education of nonprofessional food handlers, especially in the food service industry and in homes; whether this will be achieved remains to be seen. The failure of these individuals to get a proper understanding of HACCP could lead to its failure.
- To be effective, this concept must be accepted not only by food processors but also by food inspectors and the public. Its ineffective application at any level can be detrimental to its overall success for a product.
- It is anticipated that experts will differ as to whether a given step is a CCP and how best to monitor such steps. This has the potential of eroding the confidence of others in HACCP.
- The adoption of HACCP by industry has the potential of giving false assurance to consumers that a product is safe, and therefore, there is no need to exercise the usual precautions between the purchase and consumption of a product. Consumers need to be

informed that most outbreaks of foodborne illness are caused by errors in food handling in homes and food service establishments and that no matter what steps a processor takes. HACCP principles must be observed after foods are purchased for consumption (Jay, 2000).

2.9.6. HACCP Systems in Abattoirs

Preventive systems also will include new developments such as predictive microbiology especially at CCPs (Bratchel, *et al*., 1989). The quality of a product will become less dependent upon microbiological testing and more and more upon knowledge, design and logistics of the total systems for production, processing, and distribution chain. In addition, control will be exercised through predictions based on mathematical modeling at critical control points. However, microbiological tests which can be adapted to the technology and logistics of specific production systems. It is clear that traditional microbiological approaches cannot meet these exacting requirements (Huis in't veld, *et al*., 1994).

It is important to control pathogenic microorganisms in the live animal, not only those which adversely affect the health status of the animal but also those which adversely affect the health status of the animal but also those which may cause illness in humans by transmission from foods of animal origin. At the later stages in the meat production chain, the main focus should be directed towards the prevention of contamination during the slaughter process. Total viable counts or counts of Enterobacteiaceae provide information on the hygiene of the manufacturing practices exercised and consequently about the safety and quality of the final product. Microbiological control, therefore, must include rapid, simple and cheap detection or monitoring systems for pathogenic microorganisms, as well as methods for assessing hygiene during slaughter and processing. Critical control points must be monitored, not only visually but also bacteriologically, and either consciously or periodically to ensure that these are under control (NRC, 1985). For monitoring the critical points in the slaughter and processing line it is not necessary to search for specific pathogens. At this stage of the meat production line, it is more important and makes more sense to lower the general level of contamination. In ideal situation, the absence of pathogenic microorganisms in herds should have been established before slaughter and the logistics of the process should first allow slaughtering of (specific) pathogenic-free

animals in order to avoid cross contamination on the pathogenic microorganisms often have a highly heterogeneous distribution on carcasses, very difficult at this stage (Mossel, 1982).

2.10. The Hygienic Efficiency of Conventional and Inverted Lamb Dressing System

The major slaughter line sources of microbial contamination are: fleece, Workers' hands, fecal pellets, knife blades. Aerobic Plate Counts (APC 37 ^{0}C) exceeding log 4.4 CFU cm $^{-2}$ were considered indicative of direct fleece contact, whereas *E. coli* numbers exceeding log 3.3 CFU cm^{-2} was considered indicative of direct fecal contact. A 44 ^{0}C water hand rinse removed 90% of the microbial contamination from workers' hands, but rinsed hands particularly those contacting the fleece, still carried a microbial population exceeding log 4.0 CFU cm^{-2}. A 44 ^{o}C rinse followed by 82 ^{0}C water dip reduced the contamination on knife to less than log 3.0 CFU cm^{-2}. Inverted dressing systems produced carcasses with a lower contamination level than conventional systems. With both systems little increase contamination occurred after pelt removal. The areas of highest contamination were the forequarter regions with inverted dressing. In both cases these regions are the sites where cuts are made through the skin, with both systems contamination around these cuts was entirely consistent with direct fleece contact resulting from 'rollback' (Bell and Hathaway, 1996).

In healthy slaughter animals the tissues that will ultimately become 'meat', including muscle, fat and offals, are generally regarded as being sterile (Nottingham, 1982). The contamination of these tissues with microorganisms after slaughter is undesirable but unavoidable consequence of the process by which live animals are converted into meat for human consumption. The objectives of meat hygiene are firstly to minimize contamination, to assure wholesomeness and to reduce the public health hazard posed by possible introduction of pathogens bacteria such as *Salmonella spp., Campylobacter spp. and Listeria spp*. the growth of that contaminating micro flora. Rapid cooling of the carcasses prevents the multiplication of mesophilic pathogens on the carcasses surface, thereby reducing consumer exposure and risk. Chilling also maintains wholesomeness by eliminating the growth of spoilage organisms. Today the improved health status of slaughter stocks has allowed the latter to assume equal, if no greater, importance than the former (Bell and Hathaway, 1996).

During slaughter and dressing, the major sources of microbial contamination are the slaughter animals themselves, the process workers and the processing environment. Animal Sources of carcasses contamination include the skin and the gastrointestinal and respiratory tracts (Ayres, 1955; Founard, *et al*., 1978; Grau, 1979). Contacts between the carcass and the skin, including the fleece in the case of sheep and lambs, allows contamination with a mixture of microorganisms derived from the animals pre- slaughter environment, including those fecal, soil, water and feed origin. In modern processing the respiratory and intestinal tracts are not major sources of contamination. Evisceration can be carried out with minimal contamination of the carcass (Nottingham, *et al*., 1974; Grau, 1979).

Mechanized dressing should reduce carcass contamination by limiting the alternate handling of skin and carcass by many pairs of hands as invariably occurs during manual operations (Nottingham, *et al*., 1974), although redistribution rather than an over all reduction of contamination has been reported with automated dressing (Whelehan, *et al*., 1986). On the other hand reduced carcass contamination has been reported with respect to mechanized inverted dressing of sheep carcasses (Longdell, 1992). The inverted ovine dressing system was developed over a decade ago to reduce labor costs (Longdill, 1984). The hygienic status of the carcasses was also improved since there is a reduction in clearing the hind legs and pelting down the back (Longdell, 1992).

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in selected export abattoirs located at Debre Zeit ($8^0$35' N and $39^0$06'E) and Modjo ($8^0$44'N and $38^0$58'E) towns, East Shoa Zone of Oromia Regional State.

3.2 Sampling Design

Simple random sampling was designed to find out, whether significant difference in total bacterial load (aerobic mesophilic bacteria) and total coliform counts exists between sampled goat carcasses at brisket and peri-anal area, as well as knife blades for ripping and evisceration before and after washing.

Total aerobic mesophilic bacteria and coliform counts were performed on knife blades before and after sterilization in hot water at 82 ^{0}C to find out the reduction of microbial load.

3.3 Sample Collection

3.3.1 Carcasses

Carcasses selected randomly were swabbed by placing sterile template 5 x 5 cm on selected sites of goat carcasses. A sterile cotton swab fitted with shaft, was first soaked in 10 ml of maximum recovery diluents, (MRD) (OXOID-CM 733) rubbed first horizontally and then vertically several times on the carcasses. On completion of the rubbing process, the shaft was broken by pressing it against the inner wall of the test tube and disposed, while leaving the cotton swab in the test tube. A second dry sterile cotton swab shaft fitted was taken and rubbed as before over the entire test area. On completion of the rubbing process, the shaft was broken by pressing it against the inner wall of the test tube and disposed, while leaving the cotton swab in the test tube. 0.05% sodium thiosulphate were added in the maximum recovery diluents in order to neutralize the effect of chlorinated water on APC (mesophilic) and coliforms, which occurred during washing of carcasses and knives. The test tubes were capped with a stopper and placed in cool box and transported to the laboratory. Samples that were not to be processed with in 1 hour of collection were stored at 4 ^{0}C for 24 hours (ISO 17604, 2003).

3.3.2. Knife blades

The blades of knives used for ripping and evisceration on a conventional lamb dressing line were rubbed using sterile cotton swab as like as the carcass. Samples were taken directly after knife use, after washing with water at room temperature and after dipping in hot water sterilizer at 82 ^{0}C and above. Care was taken to prevent any contamination. The approximate area of the sampled knife blades were determined by tracing the out line of the knife blades onto graph paper, and counting the number of squares on the traced outline of the graph paper.

3.4. Preparation of Maximum Recovery Diluent (MRD)

Maximum Recovery Diluents (MRD) (OXOID CM 733) was prepared as recommended by the manufacturer. Briefly, 9.5 gram of MRD was dissolved in 1 liter of distilled water. This was divided into small conical flasks and sterilized by autoclaving at 121 ^{0}C for 15 minutes.

3.5. Preparation of Decimal Dilution Rates

Series of sterile test tubes were filled with 9 ml of maximum recovery diluents (MRD) and labeled 10^{-2}, 10^{-3}, 10^{-4}. With the help of sterile pipette, 1 ml is transferred from 10^{-1} test tube to test tube labeled a 10^{-2} and from this once again 1 ml was transferred to test tube labeled as 10^{-3}. Further dilutions were prepared using the same technique.

3.6. Preparation of Standard Plate Count Agar

The Standard Plate Count Agar (OXOID CM 463) was prepared according to the manufacturer's instructions. Briefly 23.5 g of the agar was suspended in 1 liter of distilled water. After boiling, the agar was dispensed in to smaller flasks and autoclaved at 121 ^{0}C for 15 minutes. The molten agar was put in water bath at 45 ^{0} C to prevent solidification. With the help of sterile pipette, 1 ml of the sample from each dilution rate was transferred to sterile Petri dishes in duplicates. Following this, 15-20 ml of molten standard plate count agar was poured in to each Petri dish. The aliquot and the agar was mixed by swirling or tilting of each plate, taking care not to form bubbles, plates were incubated at 30 ^{0} C for 72 hours. Following this, the plates were taken out and the aerobic mesophilic bacteria colony units formed were counted.

3.7. Preparation of MacConkey Agar No-3

MaConkey Agar No-3 (OXOID CM 115) was prepared according to the manufacturers instructions by suspending 51.5 gm in 1 liter distilled water. The media was dispensed into smaller flasks and boiled to dissolve it completely and autoclaved at 121 ^{0}C for 15 minutes. The molten agar was put in water bath at 45 ^{0}C to prevent solidification. Series of decimal dilutions were prepared from the sample collected as indicated previously.

Following this, 1 ml aliquot from each dilutions rate was transferred to sterile Petri dishes in duplicates. Now 15-20 ml MaConkey Agar No-3 kept in water bath at 45 ^{0}C was poured in to the Petri dishes and mixed by moving five times in vertical, clockwise, horizontal and anticlockwise direction. Solidified plates were incubated at 35 ^{0}C for 18- 24 hours. Violet red colonies were considered to be coliforms. 5 representative colonies were picked up with a loop and transferred to 2% Brilliant Green Lactose Bile Broth (BGLB) to conform that the colonies formed were coliforms. The broth was incubated at 35 ^{0}C for 24 – 48 hours and examined for gas production. Gas production after 24 hours of incubation at 35 ^{0}C are sufficient evidence for the presence of Coliforms (Peng et al, 2001). 5 representative coliform colonies were tested to proof the presence of *E .coli* by conducting IMViC (Indole, Methyl red, Voges-Proskauer reaction and citrate utilization) test.

3.8. Water Samples Test

Water samples were collected using sterile test tubes directly from the mouth of water tap. Before taking samples the water was allowed to flow freely. Aerobic Mesophilic bacteria and coliforms counts were determined using Standard Plate Count Agar (SPA) and MacConkey agar No-3, respectively. 1 ml of the water was transferred to sterile Petri dishes in duplicates. The SPA and MacConkey agar No-3 molten agar (45^{0}C) was poured to each Petri dish, respectively. The agar and the water was mixed together well by moving five times in a vertical, clockwise, horizontal and anticlockwise direction. The plates were incubated at 30 ^{0}C for 72 hours and at 35 ^{0}C for 18-24 hours, respectively and aerobic mesophilic bacteria and coliforms were counted.

3.9. Antimicrobial Susceptibility Test

E. coli isolated from the coliforms, found from the goat carcasses in the four abattoirs were streaked on nutrient agar plate (OXOID CM 3) and incubated at 35 ^{0} C for 18 hours. Using sterile loops 4-5 well-isolated colonies were inoculated into tubes containing 4-5 ml of tryptone soya broth (OXOID CM 129). The inoculated broth was incubated at 35-37 ^{0}C for 2-8 hours, until a slight visible turbidity appeared. The turbidity was compared with 0.5 McFarland turbidity standards by holding the sample against a white back ground with contrasting black lines, until the turbidity of the suspension equated to that of the turbidity standard. The McFarland 0.5 turbidity standard was always agitated before use.

A sterile, non-toxic swab on an applicator stick was dipped in to the sample containing *E. coli*. Excess fluid was pressed out by rotating the swab firmly against the inside of the tube above the fluid level. The swab was then streaked in three directions over the entire surface of the Muller Hinton agar (OXOID CM 337) to obtain a uniform inoculation. A final sweep was made against the agar around the rim of the Petri dish.

The inoculated plates were set-aside for 5 minutes and antimicrobial discs were placed on to the top surface of Muller Hinton agar using sterile forceps. The plates were incubated at 35 ^{0}C for 18 hours in inverted position. Following this, the diameters of the inhibition zones were measured using a caliper in mm. The results were reported as susceptible, moderately susceptible, intermediate or resistant (NCCLS, 1990).

3.10. Statistical Analysis

The number of colony forming units (CFU) per Centimeter Square of sample was calculated using the following formula recommended in ISO 4833 (ISO 48 33, 1991).

$$\mathbf{N} = \frac{\text{SUM C}}{(1 \times n_1) + (0.1 \times n_2) \times d}$$

Where N = number of colonies per ml sample

SUM C = Sum of colonies on all plates counted

n_1 = number of plates in first dilution counted

n_2 = number of plates in second dilution counted

d = dilution from which the first count are obtained

A) 10N/ 25 CFU per square centimeter of sampled carcass surface

B) 10N/59.475 per square centimeter of sampled knife surface

The results of microbial counts (CFU) were first transformed to logarithms ($\log_{10}$) values and the transformed values were analyzed using SPSS –11.5 software.

4. RESULTS

4.1. Mean AMBC Count Before and After Washing

The study showed less mean AMBC count in abattoir "X", "Y" and "Z" after washing in the brisket area, but more count was observed after washing in abattoir " W". The mean AMBC count of the peri-anal area reduced in all the abattoirs after washing .The knife blades used for ripping and evisceration had less mean AMBC count after washing with water at room temperature and after dipping in hot water sterilizer at 82 ^{0}C and above in all the four abattoirs (Table 4.1.).

Table 4.1. Mean Aerobic Mesosphilic Bacteria Count ($\log_{10} Cm^{-2}$), 95%CI

No	Sampling area	Abattoirs											
		W			X			Y[1]			Z		
		N[5]	Mean	SD[6]	N	Mean	SD	N	Mean	SD	N	Mean	SD
1	Brisket before washing	16	5.94	0.074	16	5.93	0.067	14	5.87	0.034	16	5.88	0.077
2	Brisket after washing	16	5.96	0.084	16	5.04	1.41	12	5.67	0.064	16	4.78	1.49
3	PA[2] before washing	16	5.97	0.067	12	6.01	0.022	15	5.90	0.030	16	5.89	0.061
4	PA after washing	16	5.86	0.041	16	5.91	0.080	16	5.58	0.056	15	5.84	0.054
5	KR[3] before washing	16	5.58	0.068	16	5.61	0.049	15	5.49	0.031	16	5.51	0.074
6	KR after washing	16	5.47	0.055	16	5.53	0.070	16	2.20	0.154	16	5.39	0.092
7	KE[4] before washing	16	5.59	0.071	16	5.598	0.065	15	5.52	0.029	16	5.50	0.046
8	KE after washing	16	5.49	0.90	16	5.54	0.073	16	2.17	0.154	14	5.40	0.042

1-Abattoir Y used 82^{0}C hot water sterilizer for knives, 2-= Peri-anal area, 3= Knife used for Ripping, 4 =Knife used for Eviscerations,
5 = Number of samples, 6 = Standard Deviation

4.2. Mean Coliform Count Before and After Washing

Mean Coliform count was more in all the four abattoirs in the brisket area after washing than before washing, while the mean count was less in the peri- anal area in the four abattoirs after washing. Knife blades used for ripping and evisceration had less mean coliform count after washing with water at room temperature and after dipping in hot water sterilizer at 82 ^{0}C and above in all the four abattoirs (Table 4.2.).

Table 4. 2. Mean Coliform Count ($\log_{10} Cm^{-2}$), 95% CI

NO	Sampling area	Abattoirs											
		W			X			Y[1]			Z		
		N[5]	Mean	SD[6]	N	Mean	SD	N	Mean	SD	N	Mean	SD
1	Brisket before washing	16	2.86	0.078	16	2.87	0.060	16	2.80	0.056	15	2.77	0.064
2	Brisket after washing	16	2.99	0.86	16	2.88	0.066	16	2.84	0.075	15	2.80	0.090
3	PA[2] before washing	16	2.90	0.089	16	2.89	0.043	15	2.81	0.038	15	2.82	0.059
4	PA after washing	15	2.89	0.052	16	2.82	0.059	16	2.71	0.097	16	2.81	0.105
5	KR[3] before washing	16	2.49	0.086	16	2.54	0.067	15	2.40	0.045	15	2.44	0.062
6	KR after washing	16	2.39	0.079	16	2.434	0.059	16	2.05	0.220	16	2.333	0.124
7	KE[4] before washing	16	2.50	0.084	16	2.536	0.079	15	2.44	0.032	15	2.42	0.047
8	KE after washing	16	2.43	0.110	16	2.45	0.047	16	1.94	0.272	16	2.334	0.101

1-Abattoir Y used 82^{0}C hot water sterilizer for knives, 2= Peri-anal area, 3 = Knife used for Ripping, 4= Knife used for Evisceration, 5= Number of samples, 6 =Standard Deviation

4.3. Comparison of Mean AMBC Count Before and After Washing

There was statistically significant ($p<0.05$) difference in mean AMBC count before and after washing in abattoir "X", "Y" and "Z" in brisket area .The peri-anal are mean AMBC count was significantly ($p<0.05$) less after washing in all the four abattoirs. Knife blades used for ripping and evisceration showed statistically significant ($P<0.05$) difference after washing with water at room temperature and after dipping in hot water sterilizer at 82 ^{0}C and above in all the four abattoirs (Table 4. 3.).

Table 4.3. Paired Samples t Test for Comparison of Mean AMBC Before and After Treatment ($\log_{10} Cm^{-2}$), 95% CI

Pair	Sampling area	Abattoirs											
		W			X			Y[1]			Z		
		t[5]	df[6]	Sig. (2-tailed)	t	df	Sig. (2-tailed)	t	df	Sig. (2-tailed)	t	df	Sig. (2-tailed)
1	Brisket before & after washing	-1.644	15	.121	2.575	15	.021	12.19	11	0.000	2.894	15	.011
2	PA[2] before & After washing	6.546	15	0.000	5.307	11	0.000	32.23	14	0.000	3.646	14	0.003
3	KR[3] before & After washing	7.055	15	0.000	4.701	15	0.000	87.17	14	0.000	7.538	15	0.000
4	KE[4] before & After washing	6.229	15	0.000	3.619	15	0.003	83.68	14	0.000	8.148	13	0.000

1-Abattoir Y used 82^{0}C hot water sterilizer for knives, 2-= Peri-anal area, 3= Knife used for Ripping, 4= Knife used for Evisceration,
5= t value, 6= degree of freedom

4.4. Comparison of Mean Count of Coliforms Before and After Washing

Mean coliform count in brisket area showed statistically significant (P<0.05) difference, before and after washing in abattoir "W" and "Y". The peri-anal area mean coliform count was significantly (P<0.05) less after washing in abattoir "X" and "Y". Knife blades used for ripping and evisceration showed significantly (P<0.05) less mean count of coliforms after washing with water at room temperature and after dipping in hot water sterilizer at 82 ^{0}C and above in all the four abattoirs (Table 4. 4).

Table 4.4 Paired Samples t test for Comparison of Mean Coliform Count Before and After Treatment ($\log_{10} Cm^{-2}$), 95% CI

Pair	Sampling area	Abattoirs											
		W			X			Y[1]			Z		
		t[5]	df[6]	Sig. (2-tailed)	t	df	Sig. (2-tailed)	t	df	Sig. (2-tailed)	t	df	Sig. (2-tailed)
1	Brisket Before & after washing	-12.425	15	0.000	-.173	15	.865	-4.26	15	0.001	1.656	13	0.122
2	PA_2 before & After washing	-.068	14	.947	5.182	15	0.000	5.272	14	0.000	.504	14	0.622
3	KR[3] Before & After washing	9,744	15	0.000	7.042	15	0.000	6.484	14	0.000	5.039	14	0.000
4	KE[4] Before & After washing	5.648	15	0.000	4.967	15	0.000	7.233	14	0.000	5.555	14	0.000

1-Abattoir Y used 82^{0}C hot water sterilizer for knives, 2= Peri-anal area, 3= Knife used for Ripping, 4 =Knife used for Evisceration,
5= t value, 6- df =degree of freedom

4.5 Comparison of Mean Microbial Count on Knife Blades Before and After Treatment

Reduction of Mean AMBC and Coliform count was observed on knife blades used for ripping and evisceration after washing with water at room temperature, but the reduction rate was greater after dipping in hot water knife sterilizer at 82 ^{0}C and above (Table 4.5).

Table 4.5 Comparison of Mean Bacterial Reduction Rate of Knife Blades Before and After Treatment

Type of microbes	Sampling area	Before washing in abattoirs ($\log_{10}$ cm^{-2})				After washing in abattoirs ($\log_{10}$ cm^{-2})				Reduction rate in abattoirs (%)			
		W	X	Y	Z	W	X	Y	Z	W	X	Y	Z
AMBC[1]	KR[2]	5.58	5.61	5.49	5.51	5.47	5.53	2.20	5.39	22.37	51.02	99.95	24.14
	KE[3]	5.59	5.598	5.52	5.50	5.49	5.54	2.17	5.40	20.57	12.50	99.96	20.57
Coliform	KR	2.49	2.54	2.40	2.44	2.39	2.434	2.05	2.333	20.57	21.65	55.33	21.84
	KE	2.50	2.536	2.44	2.42	2.43	2.45	1.94	2.334	36.90	17.96	68.41	17.70

1= Aerobic Mesophilic Bacteria Count, 2=Knife used for Ripping, 3= Knife used for Evisceration

Out of the 1280 representative colonies of coliforms which were tested for gas production by 2% Brilliant Green Lactose Bile Broth (BGLB) and tested again for IMViC (Indol, Methyl red , Vogues Proskauer reaction and Citrate utilization test) 90.36% were *E. coli.*

4.6 Water Samples Test

Out of the 64 water samples tested, 12 (18.75%) were coliform positive and aerobic mesophilic bacteria were observed in 25 (39.06%) of the samples (Figure 4.1 and 4. 2)

Figure 4.1. Water Samples Coliform Count

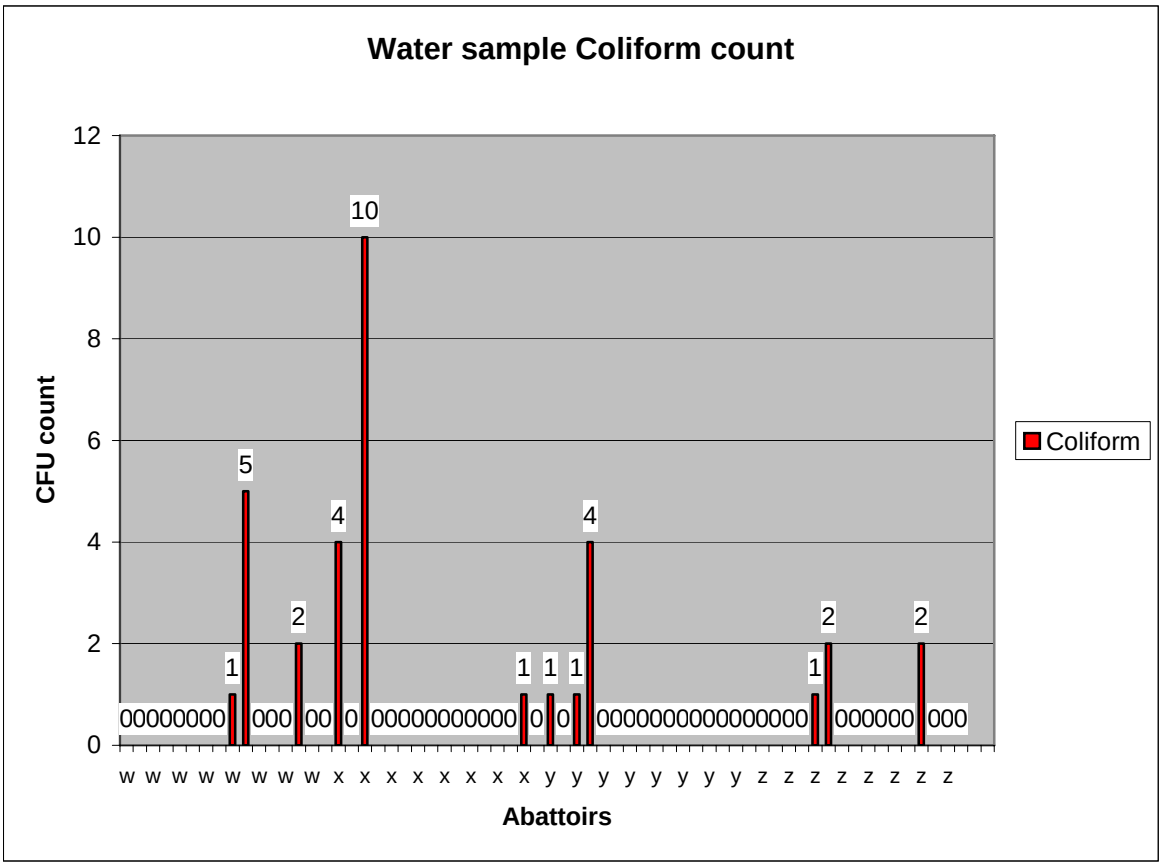
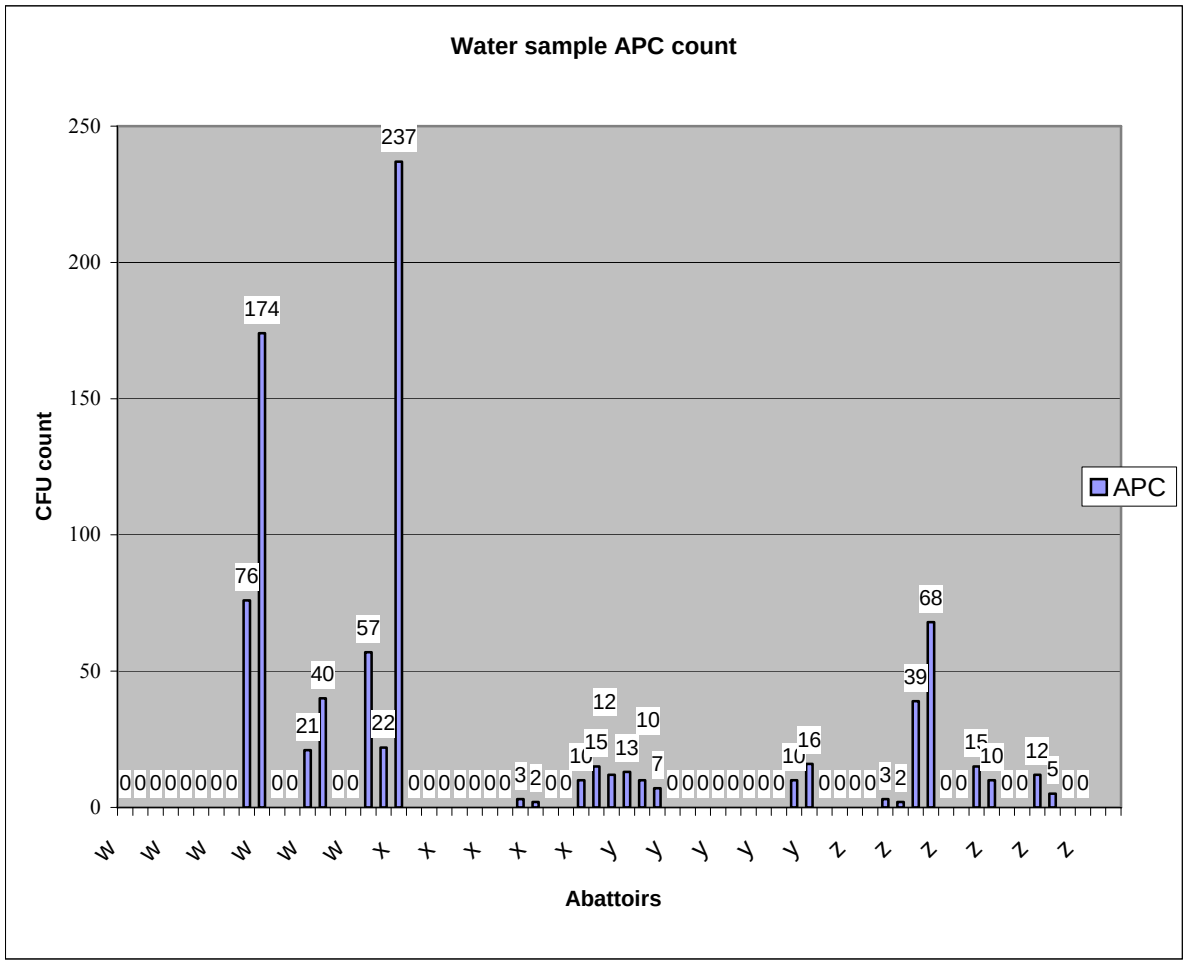


Figure 4.2. Water Samples APC Count



4.7 Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was carried out after isolation of 20 *E. coli* strain from the coliforms, found from goat carcasses which were collected from the four abattoirs. The 20-isolated *E. coli* strain was tested for their antimicrobial susceptibility using antibiotics commonly available. Antimicrobial susceptibility test of *E. coli* did not show any significant resistant to the tested antimicrobial agents (Table 4.6 and 4.7).

Table 4.6 Antibiogram of *E. coli* Isolates

Plate no of *E.coli* Isolates	Diameter of zone of inhibition of antibimicrobial agents (mm)											
	Co[1]	Rif[2]	Ery[3]	T[4]	S[5]	K[6]	Clt[7]	Va[8]	C[9]	Cx[10]	Ac[11]	Pen[12]
1	26	6	6	20	18	20	16	6	25	6	23	6
2	27	6	9	24	17	21	15	6	28	6	21	6
3	27	6	12	21	18	20	16	6	27	6	22	6
4	27	6	13	23	17	22	16	6	28	6	22	6
5	26	6	11	21	18	22	17	6	29	6	22	6
6	29	6	6	18	16	19	15	6	29	6	24	6
7	27	6	6	18	15	19	16	6	29	6	24	6
8	24	6	6	22	15	20	15	6	25	6	25	6
9	27	6	6	20	15	19	15	6	23	6	23	6
10	26	6	9	23	19	22	15	6	28	6	20	6
11	26	6	6	20	15	18	16	6	27	6	22	6
12	27	6	6	18	15	19	15	6	27	6	21	6
13	27	6	6	22	15	19	16	6	26	6	23	6
14	26	6	6	19	15	18	15	6	28	6	22	6
15	27	6	6	20	16	18	15	6	27	6	22	6
16	26	6	6	19	15	18	15	6	26	6	20	6
17	27	6	6	21	15	18	16	6	26	6	23	6
18	26	6	6	21	15	18	15	6	28	6	22	6
19	26	6	6	20	15	18	16	6	27	6	22	6
20	26	6	6	18	15	18	15	6	26	6	22	6

1= Co trimoxazole, 2= Rifampicin, 3= Erythromycin, 4= Tetracycline,5= Streptomycin,6= Kanamycin,7= Cephalothin, 8= Vancomycin , 9= Chlorapmhenicol, 10= Cloxacillin, 11= Amoxyclav, 12= Penicillin

Table 4.7 Antimicrobial Agents Used to Test Susceptibility of *E. coli* Isolates

Antimicrobial agents	Amount/ disk (μg)	Resistant	Moderately Susceptible	Susceptible
Penicillin	30	√		
Vancomycin	30	√		
Cloxacillin	5	√		
Erythromycin	15	√		
Rifampicin	5	√		
Cephalothin	30		√	
Streptomycin	10			√
Kanamycin	30			√
Tetracycline	30			√
Amoxyclav	30			√
Cotrimoxazole	25			√
Chloramphenicol	10			√

5. DISCUSSION

Minimizing bacterial contamination of carcasses during and immediately after slaughter improves the microbiological safety of meat. Most bacterial contamination of carcass surfaces occurs during slaughter and/ or dressing procedures from a variety of sources, such as hides, intestinal contents, contact surfaces and personnel handling of the carcass (Hanson, *et al.,* 1990).

Contamination of carcasses occurs as a result of direct contact with contaminated material such as knives, workers' hands, fleeces and fecal materials. The results of this study showed a mean AMBC count 5.58, 5.61, 5.49, and 5.51 log $_{10}$ CFU cm $^{-2}$ in abattoir "W", "X","Y" and "Z", respectively on the knife blades used for ripping before washing. The mean count decreased to 5.47, 5.53, 5.39 log $_{10}$ CFU cm $^{-2}$ in abattoir **"**W", "X" and "Z", respectively after washing with water at room temperature. This accounts for 22.37% and 51.02 and 24.14% reduction rates in abattoir "W", "X", and "Z", respectively. The mean AMBC count of abattoir "Y" reduced to 2.20 log $_{10}$ CFU cm $^{-2}$ after dipping in a hot water knife sterilizer at 82 ^{0} C and above, which accounts for 99.95 % reduction rate.

The mean AMBC count of knife blades used for evisceration before washing were 5.59, 5.598, 5.52 and 5.50 log $_{10}$ CFU cm $^{-2}$ in abattoir "W", "X", "Y", and "Z", respectively. The mean count decreased to 5.49, 5.54 and 5.40 log $_{10}$ CFU cm $^{-2}$ after washing with water at room temperature in abattoir "W", "X", and "Z". This accounts for 20.57, 12.50, and 20.57% reduction rate in the given order, but the mean count reduced to 2.17 log $_{10}$ CFU cm $^{-2}$ after dipping in hot water knife sterilizer at 82 ^{0}C and above. In abattoir "Y" the reduction rate was 99.96%.

The mean coliform count of knife blades used for ripping before washing were 2.49, 2.54, 2.40, 2.44 log $_{10}$ CFU cm $^{-2}$ in abattoirs "W", "X", "Y" and "Z" respectively. The mean counts decreased to 2.39, 2.434, and 2.333 log $_{10}$ CFU cm $^{-2}$ after washing with water at room temperature in abattoir "W", "X" and "Z", respectively, which accounts for 20.57, 21.65, and 21.84% reduction rate. The mean coliform counts of abattoir "Y" reduced to 2.05 log $_{10}$ CFU cm -^{2} after dipping the knife blades in hot water sterilizer at 82 ^{0}C and above. This accounted for 55.33% reduction rate.

The mean coliform count of knife blades used for evisceration before washing were 2.50, 2.536, 2.44 and 2.42 $\log_{10}$ CFU cm^{-2} in abattoir "W", "X", "Y" and "Z". The mean count decreased to 2.43, 2.45, and 2.334 $\log_{10}$ CFU cm^{-2} in abattoir "W", "X", and "Z", respectively, which accounts 36.90, 17.96 and 17.70% reduction. The mean coliform counts of abattoir "Y" reduced to 1.94 $\log_{10}$ CFU cm^{-2} after dipping in hot water knife sterilizer at 82 ^{0}C and above which accounts for 68.41% reduction.

The reduction of mean AMBC and coliform counts on knife blades used for ripping and evisceration after washing showed significant ($p<0.05$) difference for all the four abattoirs. These results showed washing with water at room temperature decreased mean AMBC and coliforms count. Dipping knife blades in hot water knife sterilizer at 82 ^{0}C and above in abattoir "Y" reduced the mean AMBC and coliforms counts greatly. The mean AMBC count was reduced by 99.9%, while the mean coliform counts was reduced to 55.33 and 68.41%. The low reduction of mean coliform count compared to mean AMBC count after dipping knife blades in hot water sterilizer at 82 ^{0}C and above in abattoir "Y" could be attributed to the duration of sterilization time and to too low level of hot water sterilizer. Conventional 82 ^{0}C water treatment combined with a 44 ^{0}C spray rinse results in a 99.8% reduction in the microbial contamination carried on a knife blades (Bell and Hathaway, 1996).

After washing, the mean AMBC count in brisket area on the carcasses decreased from 5.93, 5.87, and 5.88 $\log_{10}$ CFU cm^{-2} to 5.04, 5.67 and 4.78 $\log_{10}$ CFU cm^{-2} in abattoir "X", "Y", and "Z", respectively, but the mean count increased from mean 5.94 $\log_{10}$ CFU cm^{-2} to 5.96 $\log_{10}$ CFU cm^{-2} $\log_{10}$ in abattoir "W". These difference in counts were significant ($p<0.05$) for abattoir "X", "Y", and "Z". After washing the mean AMBC count of peri-anal area on the carcasses decreased from mean 5.97, 6.01, 5.90 and 5.89 $\log_{10}$ CFU cm^{-2} to 5.86, 5.91, 5.58 and 5.84 $\log_{10}$ CFU cm^{-2} in abattoir "W", "X", "Y" and "Z", respectively. The differences between the mean counts were significant ($p<0.05$) in all the four abattoirs.

After washing the mean coliform count in brisket area on the carcasses increased from 2.86, 2.87, 2.80 and 2.77 $\log_{10}$ CFU cm^{-2} to 2.99, 2.88, 2.84 and 2.80 $\log_{10}$ CFU cm^{-2} in abattoir "W", "X", "Y" and "Z", respectively. The difference between the means was significant ($p<0.05$) for

abattoir "W" and "Y". After washing, the mean coliform count in peri-anal area on the carcasses decreased from 2.90, 2.89, 2.81 and 2.82 $\log_{10}$ CFU cm^{-2} to 2.89, 2.82, 2.71, and 2.81 $\log_{10}$ CFU cm^{-2} abattoir "W", "X", "Y" and "Z", respectively. The differences between the mean counts were significant ($p < 0.05$) in abattoirs "X" and "Y". The mean APC counts at brisket and peri-anal area of the sampled carcasses decreased after washing in all abattoirs, except for abattoir "W" where an increased AMBC count was recorded in brisket area .The mean coliform counts in brisket area of the sampled carcasses increased after washing while at peri-anal area decreased.

These results showed that after washing increment of mean coliform count at brisket site and decrement at perianal area. This indicates that under the conventional dressing system, coliform from highly contaminated peri anal area were carried down with wash water along the carcass to the less contaminated brisket area. This might be due to the less time spent on washing, less volume and pressure of water or the poor design of the spraying device. Effectiveness of washing varies with the time spent on washing, volume, pressure and temperature of the water and design of the spraying device (Baily, 1971).

Comparisons between the four abattoirs showed namely, abattoirs "Y" and "Z" was better than the other two abattoirs. This was evidenced by more mean aerobic mesophlic bacterial and coliform counts in abattoirs "W" and "X".

In abattoir "Y" hot water knife sterilizer at 82 ^{0}C and above were used to sterilize the knives. It is generally accepted that the use of good manufacturing practices (GMP) at the slaughterhouse will correlate with low carcass bacterial counts. However, even under GMP's some bacterial carcass contamination inherent to slaughtering can be expected. On the other hand, contamination is primarily uneven, because of accidental contact with contaminated materials. Thus, sampling small areas will be inappropriate to determine the differences in bacterial load between abattoirs (Lasta and Fonrough, 1998). Other factors, such as time of sample taking, season and method of sample taking might affect the bacterial counts (Lasta, *et al*., 1992).

The swab method recovers only 20% of bacterial load (Ingram and simonsen, 1980). The AMBC and coliform counts in this study could be more if excision method had been applied.

The relatively high bacterial load, detected in the abattoirs could be attributed to negligence to respect GMP, in the slaughterhouse and lack of knowledge of GMP by the abattoir personnel and owners. Mostly, the butchers do not wash and sterilize their knives, wash their hands with water and detergents. Moreover, improper functioning of knife sterilizer, piling of skins on the killing floor, over crowding of animals at the bleeding site, improper cleaning of the liarage and failure to separate dirty and clean working areas contribute to high bacterial load of the carcass.

Out of the 64 water samples tested, 12 (18.75%) were Coliform positive and aerobic mesophilic bacteria was observed in 25 (39.06%) of the samples. The highest Coliform counts were 10 CFU, which was registered in abattoir "X" one time, and 5 CFU was observed in abattoir "W" one time. The other Coliform counts were less than 5 CFU and 52 of the water sample were free from Coliforms. The highest AMBC counts were 271 and 174, which were observed in abattoir "X", and "W" respectively. The water sample test was carried out in order to find out if there were significant high number of microbial colony forming units that could affect the after washing bacterial colony counts. The result showed that the after wash count could not be affected, because the counts were most of the time were nil and even some of the counts encountered could be due to contamination of the mouth of the water tap.

Anti microbial susceptibility test was carried out after isolation of 20 *E. coli* from the coliforms, which were collected from carcasses of the four abattoirs. The 20 *E. coli* isolates were tested for their antimicrobial susceptibility using antibiotics commonly available. The antimicrobial susceptibility test was perfomed to find out if there were any antibiotic strain of *E .coli*, which could have any value of clilnical significance and pose risk for the public, but the antimicrobial susceptibility test of *E.coli* did not show any significant resistant to the tested antimicrobial agents which are commonly used.

6.CONCLUSION AND RECOMMENDATIONS

The study showed sterilization of knives in hot water sterilizer greatly reduces bacterial load. The study also showed that after washing increment of mean coliform count at brisket site and decrement at peri-anal area. This indicates that under the conventional dressing system, coliform from highly contaminated peri-anal area were carried down with wash water along the carcass to the less contaminated brisket area. The increment of coliforms in the brisket area after carcass washing may be attributed to the less volume of water utilized for washing and to the poor design of water spraying device.

Comparisons between the four abattoirs showed namely, abattoirs "Y" and "Z" was better than the other two abattoirs. This was evidenced by more mean aerobic mesophilic bacteria and coliform counts in abattoirs "W" and "X".

The microbial count of water sample showed that the after wash count could not be affected, because the counts were most of the time were nil and even some of the counts encountered could be due to contamination of the mouth of the water tap.

Antimicrobial susceptibility test of *E. coli* did not show any significant resistant to the antimicrobial agents which are commonly used.

Though microbial status of carcasses depend upon many factors such as breeding and transportation conditions of animals, good manufacturing practices (GMP) at the slaughter house, starting from lairage to cold room storage of carcasses can reduces the microbial status of carcasses to the lower level. These in turn improve the microbiological safety of meat and elongate the shelf life meat and reduce the risk posing public health hazards. Hence, in order for Ethiopian meat export abattoirs to be able to provide safe, sound and wholesome meat to the consumer the following recommendations are forwarded: -

- Encourage abattoir workers and owners to the implementation of good hygienic practice (GHP).
- Regular and frequent washing knives and their sterilization at 82 ^{0}C hot water.
- Regular and frequent washing of hands with hot water (44 ^{0}C) and detergents
- Ensuring the proper functioning of knife sterilizers.
- Avoid piling of skins on the killing floor.

- ❖ Avoid the overcrowding of the bleeding area by slaughter animals.
- ❖ Use high pressure and large volume of water with good spraying device to wash the carcasses.
- ❖ Cleaning of the liarage and the killing floor properly and regularly.
- ❖ Carry out immediate removal of waste materials from the slaughterhouses.
- ❖ Train regularly abattoir workers about hygiene and hygienic handling of carcasses and meat contact surfaces.
- ❖ Separate the dirty and clean working areas.
- ❖ Introduce the application of HACCP in abattoirs to monitor the hygienic production of fresh meat.
- ❖ Carry out further survey on total bacterial, Coliform and *E. coli* counts on carcasses of other food animals.

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SIGNED DECLARATION SHEET

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

Name: Tesfaye Mengistu Teka

Signature.....

Date of submission.....

This thesis has been submitted for the examination with my approval as advisor.

Dr. Girma Zewde Signature _____

(Associate professor)